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How Do the Largest and Smallest Baboon Species Compete for Reproductive Success in a Natural Hybrid Zone?

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How Do the Largest and Smallest Baboon Species Compete for Reproductive Success in a Natural Hybrid Zone?

by
Monica McDonald

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ABSTRACT OF THE DISSERTATION

How do the largest and smallest baboon species compete for

reproductive success in a natural hybrid zone

by

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Professor Jane Phillips-Conroy, Chair

This dissertation examines hybridization between two of the most divergent baboons, the kinda baboon (*Papio kindae*) and the grayfooted chacma baboon (*P. ursinus griseipes*), which differ markedly in body size and in some social behavior. Preliminary research revealed hybridization between males of the smaller species (kinda) and females of the larger species (grayfoots), but not the reverse. Using behavioral, phenotypic, and genetic data collected from a single hybrid group in Kafue National Park from May 2012 to July 2013, I evaluated whether a similar asymmetry was borne out in this group and whether phenotypic markers of species assignation matched genotypic groupings based upon seven microsatellite markers. I assessed what factors were influential for male mating success in this group and explored whether mating and reproductive success could be explained by the priority-of-access model, whereby the dominant male realizes the most reproductive success. I investigated whether a modified form of this priority-of-access model, female preference for unusually "friendly" kinda males, and/or genetic or obstetric incompatibility might explain this proposed asymmetry. I found that while asymmetry is present in the overall hybrid zone, it was not found in this group. Phenotypic

markers of species assignation did not match genotypic groupings. As in most other baboon species, dominance rank and mate guarding were the most influential factors in male reproductive success in this group, supporting the priority-of-access model. However, since Kinda-like males in this group groom more and have higher reproductive success, the asymmetry may be in part due to this “friendly” behavior. While mating occurred across all genotypes and phenotypes, the lack of hybrid offspring resulting from parents having opposite genetic backgrounds suggests possible genetic or obstetric incompatibility. Results from this study reveal that being a genetically Kinda-like male (regardless of phenotype) confers some sort of reproductive advantage. This study has helped clarify the importance of "friendly" male behavior in this unexpected asymmetry, provided insight into issues related to mate choice in hybrids, and revealed possible reproductive barriers to hybridization, as well as contributed to the corpus of knowledge of baboon diversity in general.

Chapter 1: Introduction

In spite of their diversity in size, appearance and social behavior, all species of baboons (*Papio*) apparently interbreed where their ranges naturally adjoin. This study uses phenotypic, behavioral, and genetic data to examine hybridization between two of the most divergent, the kinda baboon (*Papio kindae*) (henceforth "kindas") and the grayfooted chacma baboon (*Papio ursinus griseipes*) ("grayfoots"), which differ markedly in body size and some social behaviors. This study contributes to other ongoing research investigating diversity within this genus of large, open-country primates, whose ecology, evolutionary history, and population structure show informative analogies to those of early human ancestors and relatives (Hominini) [Jolly, 2001].

Early field surveys by Rogers, Burrell, Phillips-Conroy and Jolly in Kafue National Park, Zambia [Jolly et al., 2010] identified several baboon groups that included individuals of hybrid phenotype. Genetic analyses based upon fecal samples from these groups confirmed their hybrid origin, finding mitochondrial and Y-chromosome haplotypes of both species, as well as "discordant" males, those carrying Y-chromosomes from one species, and mitochondrial haplotypes from the other. Out of 55 samples in which both mitochondrial and Y-markers were assessed, all 15 cases of "discordant" males carried kinda Y-chromosomes (patrilineal inheritance) and grayfoot mitochondria (matrilineal inheritance). This suggests that initial hybridization in this area occurred when male kindas successfully bred with female grayfoots (kinda x grayfoot), but that the reciprocal (grayfoot x kinda) cross was rarely successful [Jolly et al., 2010]. This observation differs from expectation given that male kindas, which are half of the

body mass of the grayfoot males, might be expected to lose in interspecific competition for mates.

Figure 1: Map of Zambia highlighting places mentioned throughout this dissertation. Ngoma (red dot) is the study site for this project.

This study uses a phenotypic, behavioral, and genetic approach to investigate whether an asymmetry exists in a single previously undocumented 64-member kinda x grayfoot baboon group near Ngoma, Zambia (15.96S, 25.93E) (the southern Kafue National Park headquarters) (Figure 1). The objective of the study is to explore the behavioral underpinnings of the previously observed asymmetry, interpret the behavioral findings in the context of the parents'

degree of kinda or grayfoot ancestry, and assess the success of certain parental combinations in producing offspring.

1.1 Hybrid Zones

Hybridization—interbreeding between members of genetically differentiated populations, distinct enough to be regarded as species—was thought to be anomalous in vertebrates, but recent application of molecular genetic techniques has demonstrated this is not the case. As recent work on the genomes of extinct and extant *Homo* illustrates [Green et al., 2008, 2010], features embedded in the genetic structure of a population can document a complex history of hybridization not apparent in its current biology. For instance, the phenomenon of "nuclear genetic swamping" (aka "mitochondrial capture") results in members of an otherwise typical population of one species carrying mitochondrial haplotypes related to those of a second species. This pattern, which is believed to result from strongly asymmetrical genetic introgression driven exclusively by male dispersal between sedentary female populations [Wildman et al., 2004; Zinner et al., 2009], plays a central role in genetic dispersal within the baboon populations considered in this study and forms the basis for this study's design, as discussed in the next section.

In primates and other vertebrates, hybridization occurs most readily between congeneric taxa that differ at the species (or "subspecies") level and more rarely between species of distinct genera (e.g. between baboons (*Papio*) and geladas (*Theropithecus*) [Dunbar & Dunbar, 1974; Jolly et al., 1997]). Most hybrid zones (areas in which populations of hybrid individuals predominate) are thought to originate by secondary contact between formerly isolated

populations that have become phenotypically, genetically and perhaps behaviorally distinct but not reproductively isolated [Harrison, 1990]. Hybrid zones may be highly transient or persist over many generations, with the genetic structure of its population determined by the type and intensity of natural selection (environmentally-dependent and/or environmentally-independent), the rate of migration from each of the parental forms into the zone (balanced or asymmetric), and the relative fitness of the hybrid genotypes [Barton & Hewitt, 1989; Harrison, 1990; Arnold, 1997]. The implication of these mechanistic bases suggest that either complete fusion of the parental gene-pools, or the evolution of genetic or behavioral "mechanisms" to "defend" species boundaries [Dobzhansky, 1940; Mayr, 1942, 1963] will eventually result. Through more recent studies using molecular information to reconstruct genetic and phylogeographic history, theory has been extended to include additional outcomes, including the genesis of a new hybrid species (either in addition to or in place of the parental species), or the establishment of a dynamic species boundary with ongoing, selective, gene exchange via the hybrid zone [Arnold, 1992].

Within the order Primates, hybridization has been observed in at least 8 of 132 New World species and 26 of 233 Old World species [Detwiler et al., 2005; Cortes-Ortiz et al., 2007]. Hybridization in New World species has been described to occur between species and subspecies of howler monkeys (*Alouatta*), marmosets (*Callithrix*), tamarins (*Saguinus*), and squirrel monkeys (*Saimiri*) [Silva et al., 1992; Peres & Da Silva, 1996; Mendes, 1997; Cortes-Ortiz et al., 2007]. It has been most extensively observed and studied in species of *Alouatta* (some examples of which include *A. palliata* x *A. pigra* [Cortes-Ortiz et al., 2007], *A. palliata* x *A. seniculus* [Defler, 2004], and *A. clamitans* x *A. caraya* [Aguiar et al., 2007; Agostini et al., 2008]).

In Old World Monkeys hybridization has been documented in several species and across several taxonomic levels, and hybrid zones have been documents in over 34 locations [Detwiler et al., 2005]. The most well documented cases have been in baboons (*Papio*) and guenons (*Cercopithecus*) and are most commonly examples of parapatric hybridization, whereby two species hybridize where their ranges naturally adjoin. Parapatric hybridization often leads to persistent and stable but narrow hybrid zones [Detwiler et al., 2005]. More rarely, sympatric hybridization has been documented to occur, whereby hybridization occurs between sympatric but ecologically diverse taxa and often only occurs in anthropogenically disturbed areas or in areas where members of one species have difficulty finding conspecific mates and thus "settle" for heterospecific mates [Detwiler et al., 2005]. This type of hybridization is often sporadic and rarely leads to persistent hybrid zones (although the *Cercopithecus mitis* x *C. ascanius* hybrid zone in Gombe National Park, Tanzania seems to be an exception to this) [Detwiler et al., 2005]. Some examples of this type of hybridization include hybridization between several different guenon species (*Cercopithecus mitus* x *C. ascanius* in Uganda and Tanzania [Struhsaker et al., 1988; Detwiler, 2002; Detwiler et al., 2005], *C. cephus* x *C. nictatans* in Gabon [Tutin, 1999], *C. mitis* x *C. wolfi* in Rwanda [Detwiler et al., 2005; B. A. Kaplan pers comm.], and *C. mitis* x *Chlorocebus pygerythrus* in Kenya [de Jong & Butynski, 2010]).

Several models have been used as a framework for understanding the origin, maintenance and potential fate of hybrid zones [Arnold, 1997]. In the Tension Zone model, hybrid genotypes are assumed to be intrinsically relatively unfit (due to inviability, sterility, or hybrid breakdown) but the zone may persist if the immigration of parental genes into the zone outweighs the selection against hybrid genotypes [Hewitt, 1988; Barton & Hewitt, 1989]. The zone typically

functions as a genetic sink, receiving more gene-flow than it exports and tending to become stabilized in ecologically unfavorable regions of low population density [Barton & Hewitt, 1989; Harrison, 1993; Howard, 1993]. Alternative models invoke ecological (extrinsic) factors and habitat-specific selection, which may result in localized hybrid advantage and stabilization of the hybrid zone at or near an ecotone [Moore, 1977; Arnold, 1997]. Recent interpretations emphasize the importance of considering the diversity of hybrid genotypes and their fitness [Barton & Hewitt, 1989] based on the observation that many hybrid zones are structured by a combination of ecologically-related and habitat-independent selection [Arnold, 1997]. An added complication stems from the transience of hybrid zones, which suggests that interpreting studies of hybrid zones must consider that they cannot be assumed to have reached equilibrium, a factor that is amplified by the presence of repeated, rapid, cycles of change in climates and habitats over the past few million years [deMenocal, 2011]. Such considerations play a complex role in designing studies of baboon groups for the purposes of understanding the various factors impacting the genesis and path of hybridization.

1.2 Baboon Hybridization and Sexual Selection

The genus *Papio* includes six distinct, parapatrically-distributed species: Guinea (*Papio papio*), olive (or anubis) (*P. anubis*), hamadryas (*P. hamadryas*), yellow (*P. cynocephalus*), kinda (*P. kindae*), and chacma (*P. ursinus*) baboons [Jolly, 2001; Burrell, 2009; Jolly et al., 2010; Keller et al., 2010].

Baboons provide excellent case studies for understanding hybridization and its consequences, especially in large, slow-breeding tropical African mammals (like our early

hominin ancestors). Despite their phenotypic and genetic differences, all baboon taxa apparently hybridize where their ranges meet, and genetic evidence suggests frequent and extensive hybridization throughout the history of the genus, often resulting in nuclear genetic swamping [Newman et al., 2004; Wildman et al., 2004; Burrell, 2009; Zinner et al., 2011b]. Although some minor developmental anomalies have been recorded in captive hybrid baboons [Ackermann et al., 2006], there is no evidence of lowered viability, infertility, or extensive dysgenesis in wild populations with a recent or ancient history of hybridization. Similarly, although baboons successfully occupy many different ecozones, there is little evidence that divergent ecological adaptations constrain the interactions of neighboring species [Jolly, 1993; Kamilar & Tuttle, 2006] or directly determine the dynamics of hybrid zones.

Although previous research has indicated that phenotypic and environmental differences may not constrain hybridization, behavioral differences among baboon species differ markedly, especially in the content of male-female and male-male relationships and patterns of male dispersal and maturation. Such species-specific behaviors have been shown to influence the dynamics of at least two currently active hybrid zones in Ethiopia and Kenya.

The anubis-hamadryas hybrid zone in the Awash National Park, Ethiopia is currently thought to have originated from, and been maintained by, occasional fusion of groups of both species and cross-species migration of males from both parental taxa [Nagel, 1973; Phillips-Conroy et al., 1992; Newman, 1997; Beyene, 1998; Woolley-Barker, 1999]. Immigrant hamadryas males in anubis groups attempt to follow their species-typical agenda of harem-like bonding with females, but these bonds tend to break down when the female comes into estrus, in the face of insistent, aggressive herding and grooming by anubis males [Nystrom, 1992]. Hybrids

of both sexes tend to exhibit intermediate behaviors that influence mate choice and overall mating strategies. These studies suggest that the zone is maintained by a combination of group fusion or male migration of both species into the zone and behaviorally-mediated gene flow within it [Sugawara, 1979; Phillips-Conroy & Jolly, 1981, 2004; Phillips-Conroy et al., 1991; Nystrom, 1992; Beyene, 1998; Bergman, 2000; Beehner, 2003].

In contrast, the anubis-yellow hybrid zone in Amboseli National Park, Kenya, [Samuels & Altmann, 1986; Alberts & Altmann, 2001; Tung et al., 2008; Charpentier et al., 2012] is apparently asymmetrical, maintained by immigration and successful breeding by male anubis baboons in yellow baboon groups [Samuels & Altmann, 1986]. Phenotypic and genetic studies (using microsatellite markers) suggest that this hybrid zone is narrow and that the number of hybrids has increased over time [Alberts & Altmann, 2001; Tung et al., 2008]. Male anubis and anubis-like baboons living in yellow baboon groups possess an apparent reproductive advantage over male yellow baboons [Alberts & Altmann, 2001; Tung et al., 2008], as they mature faster and disperse earlier in life, thereby allowing a longer duration and increased mating success for these individuals.

More generally, the prevalence of nuclear genetic swamping in *Papio* attests to the central role of male-male competition, and potentially female mate selection, in the evolutionary history of the genus. In active hybrid zones, males of two (or more) species, often with different behavioral strategies, compete directly within arenas also influenced by female mate choice. Every known case of "swamping" (anubis genes replacing those of yellow and hamadryas baboons; chacma genes replacing those of yellow baboons) [Burrell, 2009; Keller et al., 2010; Zinner et al., 2011b] represents a sustained, multi-generational process, in which males of the

“invading” species consistently out-competed resident males for access to resident females, and did so within the incumbents’ geographic range despite any expected preference on the part of resident females for mates of their own species.

The prevalence of "swamping" as the outcome of secondary interspecies contact suggests two questions: What species-specific physical and behavioral characteristics of males make them effective "swampers"? What is the role, if any, of female mate-choice in favoring immigrant over resident males? On present evidence, reasonable hypotheses are that species in which males primarily do not disperse (hamadryas and probably Guineas) are vulnerable to invasion, and that species with larger-bodied males (anubis and chacma) tend to invade those with smaller ones (hamadryas, Guineas and yellows). The role of female mate-choice, if any, is unclear.

This study focuses on contact between species at the extremes of the baboon size-range where "swamping" would be expected based on the assumption that genes carried by large (in this case, chacma) males invade the gene-pool of the much smaller form (kinda baboons) (Figure 2). However, evidence to date clearly shows the kinda gene-pool is not being invaded, but that instead a disproportionate number of offspring in the mixed groups in which hybrids were first formed have kinda male parentage. It is hypothesized that the behavior of kinda males towards females may have played a part in this process.

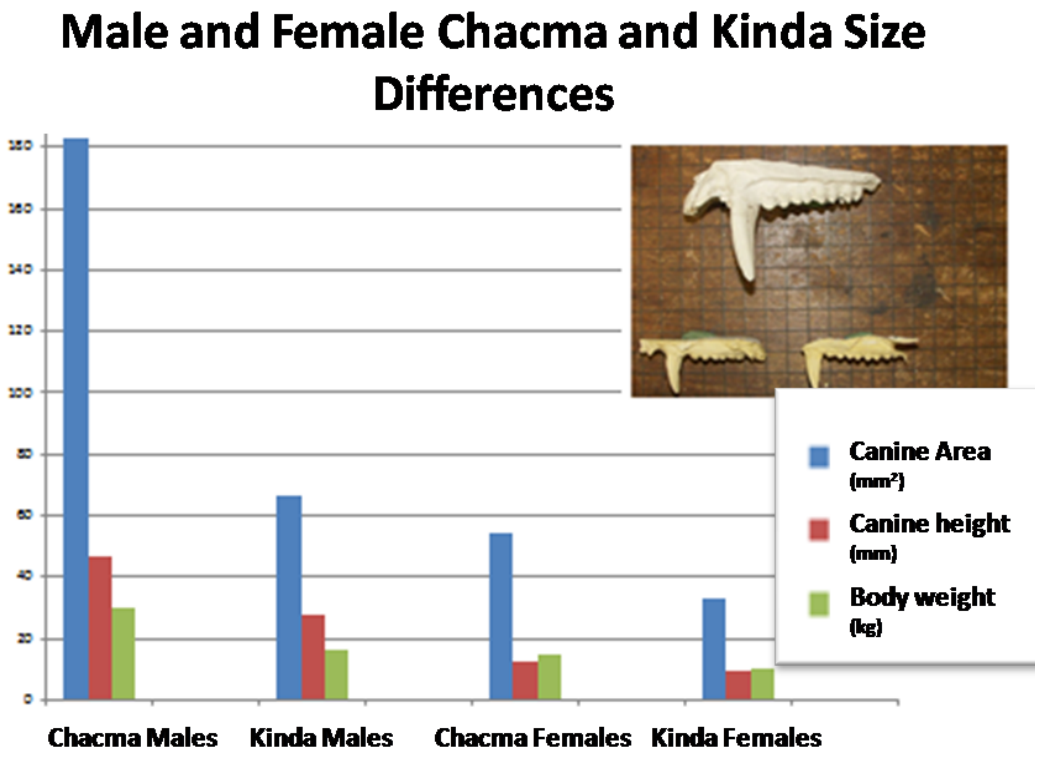


Figure 2: Comparison of dimorphic characteristics in chacma and kinda baboons; Graph courtesy of Phillips-Conroy.

1.3 Kinda and Grayfooted Chacma Baboons

Kinda baboons occur in Angola, across the northern, eastern, and central provinces of Zambia, and into the southern Democratic Republic of Congo and southwestern Tanzania [Wildman et al., 2004; Jolly et al., 2010]. Kinda baboons mostly inhabit miombo woodland habitat with greater than 1,000 mm of average annual rainfall [Ansell, 1978; Jolly et al., 2010]. Grayfoot baboons [Zinner et al., 2011a] have a more southern distribution "from the Limpopo

valley northwards through Zimbabwe, southern Mozambique, the Okavango region of Botswana, the Caprivi Strip (Namibia), and the lower Zambezi valley" [Jolly et al., 2010]. In Zambia, grayfoot baboons reside in drier miombo and mopane woodland, mostly in areas that receive less than 900 mm of average annual rainfall.

Figure 3: (a) Kinda baboons and (b) grayfooted chacma baboons. Top left (a and b): male and female body size differences. Top right (a and b): differences in female sexual swelling size and shape. Bottom (a and b): differences in pelage and appearance.

Kinda and grayfoot baboons are strikingly different in appearance (Figure 3). Kinda baboons are the smallest baboon species, with adult males averaging about 16 kg, while grayfoots are one of the largest, with adult males weighing about twice as much [Delson et al.,

2000; Jolly et al., 2010]. As predicted from their small size [Leutenegger & Cheverud, 1984], kinda baboons are also the least sexually dimorphic baboon species in body mass, with male kindas weighing on average 1.6 times as much as females (16 kg vs. 10 kg) compared to grayfoots which are among the most sexually dimorphic, with males weighing on average twice as much as females (29.9 kg vs. 14.9 kg) [Delson et al., 2000; Wildman et al., 2004; Leigh, 2006; Jolly et al., 2010] (Figure 2 and Figure 3). Kindas have soft yellow fur, long limbs, 'spectacles' of pink skin surrounding the eyes, contrasting pale cheek fur, and a characteristic mid-line crest of hair (a 'mohawk'). Female estrous swellings are comparatively small and heart-shaped (Figure 3) [Burrell et al., 2009; Phillips-Conroy et al., 2009; Jolly et al., 2010]. Grayfoots have darker pelage than kindas; they are stocky in build with large muzzles that have patches of short white fur on them; and females have large, complex sexual swellings [Burrell et al., 2009; Jolly et al., 2010] (Figure 3). Additionally, many kinda infants are born with white pelage instead of the black seen in grayfoot infants and other baboon species [Burrell et al., 2009; Phillips-Conroy et al., 2009; Jolly et al., 2010].

Almost all information about the behavior of grayfooted chacma baboons is derived from long-term studies in the Moremi Game Reserve, in the Okavango Delta, Botswana [Hamilton et al., 1976; Busse & Hamilton, 1981; Bulger & Hamilton, 1987; Cheney et al., 1996, 2004, 2006; Palombit et al., 2001, 1997, 1999, 2000; Palombit, 2003]. In social behavior, grayfooted chacmas resemble other so-called "savanna" baboons, living in multi-male, multi-female groups of variable size characterized by female philopatry and male dispersal. As in other "savanna" baboons, grayfoot societies are maintained via affiliative and agonistic social interactions such as grooming, proximity, aggression, and displacement. Grooming is typically a female activity, and

is generally female-initiated, except around the time of ovulation. Among adults, males are dominant to females, but each sex also has its own linear dominance hierarchy. Females inherit rank from their mother [Hamilton & Bulger, 1990], while adult male rank is determined by inter-individual competition [Kitchen et al., 2003, 2005]. Larger males (overall size, canine size, testicle size, etc.) tend to have higher rank. Males reach sexual maturity around 5 years of age [Smuts et al., 1987] and typically disperse from their natal group between 8 and 10 years of age [Hamilton & Bulger, 1990; Altmann & Alberts, 2003; Moscovice et al., 2009]. Females reach sexual maturity around 3 years of age [Smuts et al., 1987] and usually cycle a few times before becoming pregnant. Pregnancy lasts approximately 6 months and interbirth intervals vary from 1 to 3 years [Bulger & Hamilton, 1988]. Female reproductive cycles are 28 days; ovulation occurs 1-3 days prior to detumescence (deflation of their swelling; D-0), and it is strongly associated with maximal tumescence (peak swelling size: D-3 to D-1). The 7 days of "inflation" immediately preceding detumescence are often referred to as the “receptive or fertile period” [Smuts, 1985; Bulger, 1993; Weingrill et al., 2000]. Grayfoot societies are characterized by very strict, linear male dominance hierarchies and a lack of male-male coalitions, so that dominant males enjoy much longer consortships (3-4 days in grayfoots compared to 2-3 hours in other baboon taxa) [Smuts, 1985; Bulger, 1993; Weingrill et al., 2000] and almost exclusive mating access to estrous females during their most fertile period. However, subadult and juvenile males have been observed to mate successfully during the short interval outside of peak estrus, when the female is still partially inflated. Overall, this pattern results in a very high short-term reproductive skew [Bulger, 1993; Henzi & Weingrill, 1999; Alberts et al., 2003]. Tenure as alpha male is short (on average 6.5 months) [Palombit et al., 2000] and infanticide by incoming alpha males is frequent [Palombit et al., 1997, 2000; Henzi & Barrett, 2005; Cheney et al., 2006].

Kinda social behavior is not well documented. Preliminary research suggests both resemblances to, and significant differences from, grayfoot baboon society. Like other "savanna" baboons, kindas live in multi-male, multi-female troops that forage as coherent groups, and genetic evidence [Burrell, 2009] suggests that males disperse and females are philopatric. The most obvious difference from all other baboons so far reported—including grayfoots—is the prevalence of male-initiated grooming of adult females (Figure 4), which, rather than being associated with consortship, occurs equally across all female reproductive states [Jolly et al., 2010; Weyher et al., 2014]. Details of other aspects of behavior, such as male-male coalitions and long-term male-female "friendships", have yet to be reported.

Elaboration of secondary sexual features such as large body size, large testicles and development of large projecting canines, occurs in many species, including baboons, where competition among males for mates is high [Leutenegger & Kelly, 1977]. In females, evidence for within-sex competition is seen in the exaggerated sexual swellings and complex copulation calls, which advertise the female's time of ovulation and promote competition between males, whereby females with bigger sexual swellings and more complex copulation calls are more desirable to males [Nunn, 1999; Semple et al., 2002; Maestripieri et al., 2005]. The consequence of selection for large male body size in intra-sexual competition is that a significant degree of sexual dimorphism is found in all baboon species. This 'despotic' social system [Muller & Wrangham, 2009; Pradhan & van Schaik, 2009], with high variance in male rank and differential access to dietary and reproductive resources, sets the stage for sexual coercion. In grayfoot baboon societies this indeed appears to occur. Females have large exaggerated sexual swellings and have complex copulation calls, and large, young adult males are dominant [Bulger, 1993]

with high reproductive success [Bulger, 1993; Henzi & Weingrill, 1999; Alberts et al., 2003]. However, in other savanna baboon species, reproductive skew is less marked, as factors such as female preference [Smuts, 1985; Bercovitch, 1995; Weingrill et al., 2000], male-male coalitions [Palombit et al., 1997; Alberts et al., 2003], length of residency in the group [Smuts, 1985; Strum, 1987], and affiliative relationships between males and non-estrous females [Smuts, 1985; Palombit et al., 1997] play important roles. Females may mate with multiple males to confuse paternity, reducing the risk of infanticide and enhancing protection for themselves and their offspring [Nunn, 1999]. In Kinda baboon societies, females possess small sexual swellings and preliminary research suggests that they do not make copulation calls when mating [Chiou, 2013].

Kinda males have large testicles, which, relative to their body size are larger than those found in anubis and yellow baboons [Phillips-Conroy pers. comm.]. Males also possess sexually dimorphic canines, although canine size seems to be scaled to their smaller body size [Phillips-Conroy et al., in prep] (Figure 2). All these features are well-recognized morphological correlates of high male-male competition in baboons. The male competition/coercion model would predict that in areas where males from species with similar competitive styles but dramatically different body sizes come together and compete for females, the larger males will have higher reproductive success [Wirtz, 1999]. However, this has not been shown for kinda-grayfoot hybridization, where the smaller kinda males seem to have the reproductive advantage [Jolly et al., 2010].

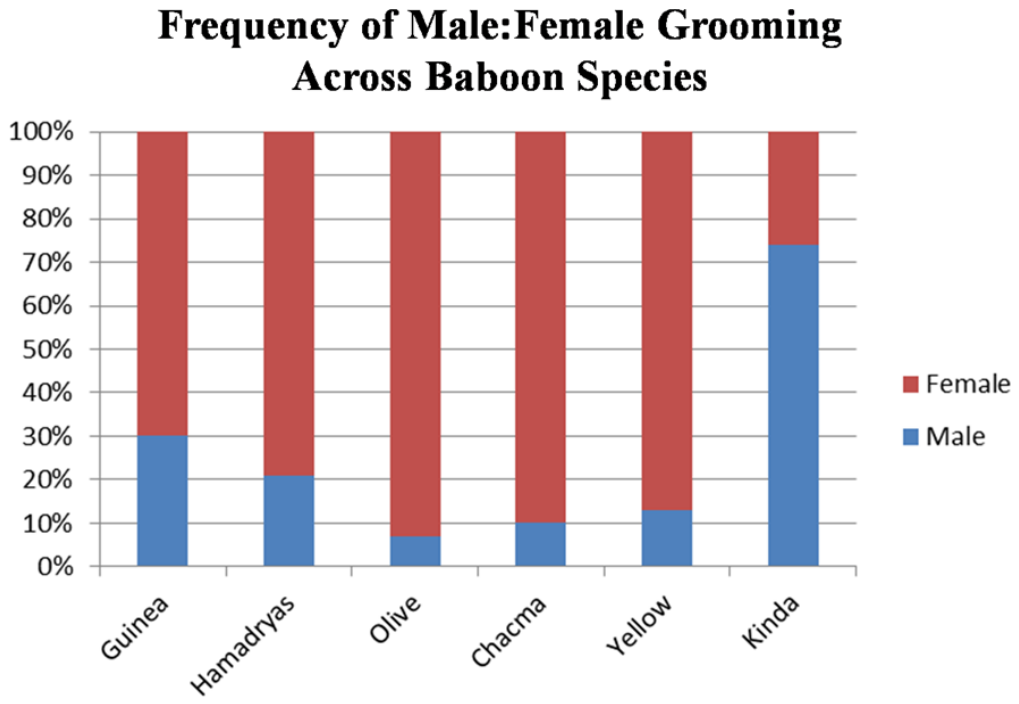


Figure 4: Intersex grooming patterns in baboons. Graph adapted from Weyher et al. [2014]. Data in this graph obtained from the following sources: Smuts [1985] (olive), Swedell [2006] (hamadryas), Nguyen et al. [2009] (yellow), Huchard et al. [2010] (chacma), Weyher et al. [2014] (kinda).

1.4 Kinda-Grayfoot Hybrid Zone in Kafue National Park, Zambia

Between 1999 and 2008 our research group conducted brief field surveys in Kafue National Park, Zambia and noted several groups of baboons that included individuals intermediate in appearance between kinda and grayfoot [Burrell, 2009; Jolly et al., 2010] (Figure 5). While marginal contact between these two species had previously been recorded in the region [Ansell, 1978], this was the first evidence of natural hybridization between them [Jolly et al.,

2010]. Analysis of fecal samples collected from these troops during these surveys provided genetic evidence for hybridization. Y-chromosomes and mitochondrial haplotypes of both parental species were present in the population. Out of 55 male samples across the hybrid zone in which both mitochondrial haplotypes and Y-markers were assessed, 15 male samples showed "Y-mitochondrial discordance". In other words, the Y-marker was inherited from one species, while the mitochondrial marker was inherited from the other [Jolly et al., 2010]. Remarkably, in all 15 cases, the Y-marker was of kinda origin and the mitochondrial haplotype was of grayfoot origin (genotype $Y_{kinda}/mt_{grayfoot}$). This included 9 cases from the "Transition Zone" (in which Ngoma is located), where individuals were noted to be hybrid in appearance, as well as 6 cases from grayfoot and kinda groups flanking the hybrid zone. Each of these cases, therefore, represented a successful mating between a kinda male and a grayfoot female somewhere in the individual's ancestry, while no case of the reciprocal cross was documented. This finding represents a significant statistical deviation from expectation [Jolly et al., 2010] and runs counter to the prediction that in species where, as in these baboons, males compete agonistically for access to females, the larger species will contribute disproportionately to the hybrid gene pool [Wirtz, 1999].

Why then, in the ancestry of this population, did kinda male x grayfoot female mating result in many hybrid descendants, while grayfoot male x kinda female mating, apparently, produced few or none? The principal aim of this research was to examine aspects of behavior that would contribute to genetic asymmetry in this group, and potentially to cast light on events that produced the observed and unexpected asymmetry in the hybrid zone. All hypotheses and predictions related to these aims are discussed in detail in Chapter 2.

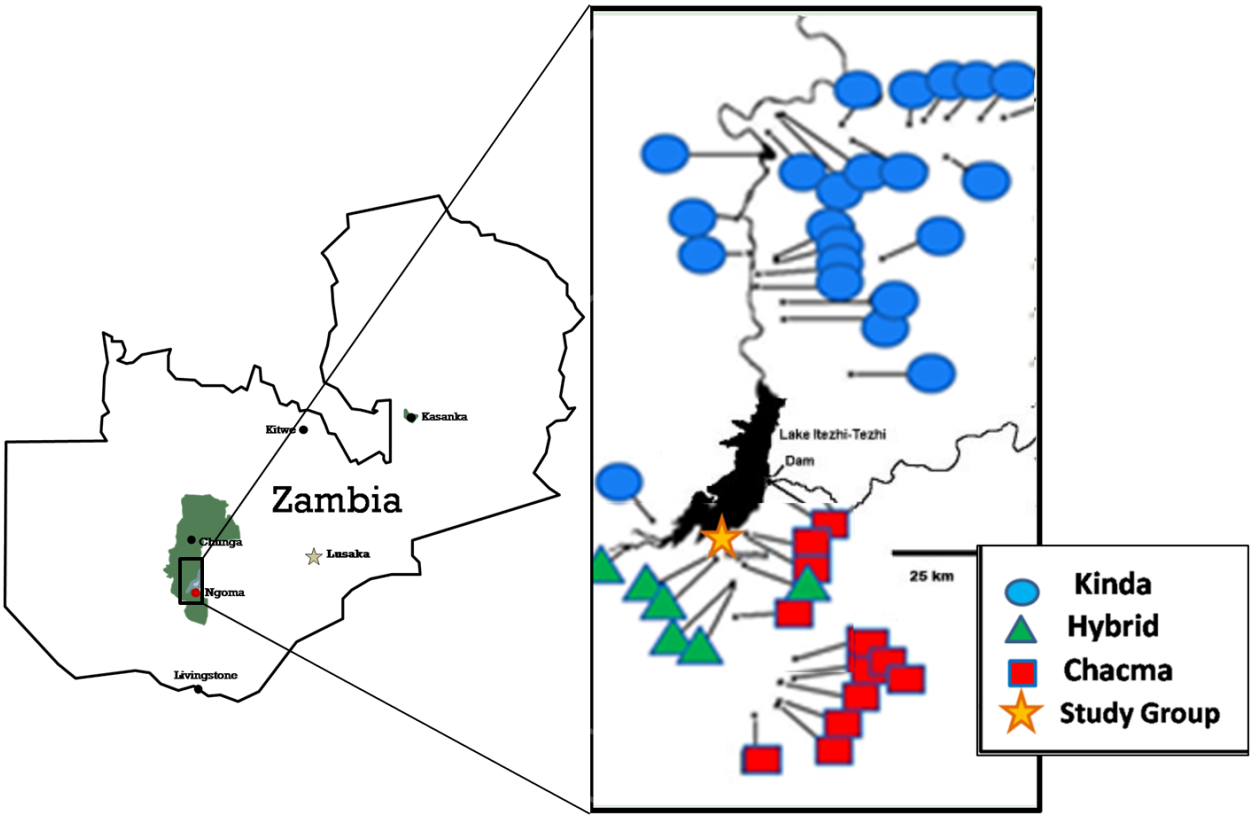


Figure 5: Observed phenotypes across kinda-grayfoot hybrid zone. Adapted from Jolly et al. [2010].

Chapter 2: Hypotheses and Predictions

Previous research by Jolly et al. [2010] revealed that a disproportionate number of hybrid descendants (n = 15) were the result of kinda males fathering offspring with grayfoot females whereas no hybrid descendents were the result of grayfoot males with kinda females. This study uses genetic markers to explore whether the same pattern is seen in a focal group of hybrid baboons. In addition, this study uses paternity and behavioral data from this hybrid group to contribute to the larger question of what factors may have led to this asymmetry.

Behavioral data from adults were collected at the same time as the fecal sample collection from infants for genetic analysis, resulting in a temporal incongruence of one birth cohort between the two types of data. However, if these observations reflect behaviors of the parental species, they would contribute to understanding events that produced hybridization in the past history of these two species. Since heritage in this dissertation is estimated on the basis of a Genotypic Hybrid Index Score (GHIS) using 7 microsatellite loci and a Phenotypic Hybrid Index Score (PHIS) based on 8 physical features, all hypotheses and predictions will be examined with regard to both measures of ancestry.

For clarity, individuals in this hybrid group that resemble one or other of the parental species are referred to as "**Kinda-like**" and "**Grayfoot-like**", respectively throughout. In later chapters (Chapters 7 and 8) that detail results using both measures of ancestry, the **phenotypes** of the individuals are denoted **Kinda-like = Kp, Intermediate**

= Ip, and Grayfoot-like = Gp, and the **genotypes** of the individuals are denoted **Kinda-like = Kg, Intermediate = Ig, and Grayfoot-like = Gg**. I present the results of the following predictions in Chapter 7 (page 134) and a discussion in the context of each of the following hypotheses in Chapter 8 (page 183).

2.1 Hypotheses and Predictions

Background: Studies of baboons elsewhere have demonstrated a suite of behavioral features relevant to mating and reproductive success [Nystrom, 1992; Bulger, 1993; Weingrill et al., 2000, 2003; Alberts et al., 2006; Tung et al., 2012]. The little that is known about kinda baboon behavior [Weyher et al., 2014], together with our extensive knowledge of chacma behavior and previous phenotypic observations and genetic studies of the kinda-grayfoot hybrid zone [Jolly et al., 2010], suggest that species differences in social behaviors (e.g. grooming, mate guarding and dominance) may have been relevant to differential reproductive success (e.g. mating success and production of offspring) in the hybrid zone. It may be that all males simply preferred to mate with grayfoot females, interpreting the larger body size and sexual swelling of grayfoot females as indicators of enhanced fertility. Or, it may be that kinda males spent more time than grayfoot males engaging in grooming and/or mate guarding behaviors which resulted in increased mating success and production of offspring.

If the latter is true, the asymmetry may have been a result of affiliative interactions between the sexes that occurred both within and outside of estrus periods. Grooming is believed to be intrinsically pleasurable for the recipient [Strum, 1987; Cheney & Seyfarth, 2007] and

grooming of females by males, rare in grayfoots but habitual in kindas, is a signal of "friendship" and is advantageous to females [Smuts, 1985; Palombit et al., 1997]. The kinda males' propensity to groom females regardless of their reproductive state, may have given them an advantage in their interactions with females. If so, this would help to explain why grayfoot females evidently allowed approach and mating by the smaller kinda males, even though the latter are small and display fewer outward signals of competitive ability (e.g., smaller canine teeth relative to grayfoots).

Alternatively, since high male dominance rank and mate guarding have been associated with high male reproductive success in baboons [Bulger, 1993; Weingrill et al., 2000, 2003; Alberts et al., 2006], it could be that dominance rank and/or mate guarding alone resulted in increased mating and reproductive success (paternity). In addition, if kinda males were able to establish dominance over grayfoot males, this may have resulted in the asymmetry.

2.1.1 Hypothesis 1: Kinda male affiliative social interactions are an adaptive reproductive strategy in hybrid zones.

This hypothesis centers on what is known about kinda behavior versus that of grayfoots. It has been argued elsewhere [Jolly et al, 2010] that the kinda male unique grooming behavior, coupled with the possible absence of competitive threat implied by their small body size, results in a reproductive advantage relative to grayfoot males in the context of the hybrid zone. Alternatively, it may be that dominance and mate guarding, both known to be significant in grayfoot male reproductive behavior, are more influential in this hybrid group. These two aspects

of kinda and grayfoot behavior are articulated in the two subhypotheses, together with their predictions, below.

Subhypothesis A: The kinda males' propensity to groom females regardless of their reproductive state may have given them an advantage in their interactions with females.

Prediction 1: In this group, males will groom females more than females groom males.

Prediction 2: Kinda-like males will groom females across all reproductive stages.

Prediction 3: Grayfoot-like males will confine their grooming to estrous females.

Prediction 4: Kinda-like males will spend significantly more time grooming females than will Grayfoot-like males.

Prediction 5: Male grooming rate of adult females will be significantly positively correlated with mating success (gauged by copulations with peak estrous females).

Since these behaviors are predicted to be important for mating access and reproductive success, the following predictions are offered:

Prediction 6: Kinda-like male copulation rate (with peak estrous females) will be significantly higher than Grayfoot-like male copulation rate.

Prediction 7: Kinda-like males will produce more offspring than Grayfoot-like males.

Subhypothesis B: Alternatively, since high male dominance rank and mate guarding have been associated with high male reproductive success in baboons, high dominance rank and/or increased mate guarding influence mating and reproductive success.

Prediction 8: Mate guarding will be significantly positively correlated with mating success (gauged by copulation with peak estrous females)

Prediction 9: Dominance rank will be significantly positively correlated with mating success (gauged by copulation with peak estrous females).

Prediction 10: The dominant male will have the highest mating success (frequency of mating with peak estrous females).

Prediction 11: The dominant male will engage in reproductive-related behaviors more than other males in the group (e.g. grooming and mate guarding).

Prediction 12: The dominant male will father the most offspring.

Two additional hypotheses are presented below for completeness, although they were unable to be completely tested given the temporal scale of this project or due to the violation of necessary assumptions.

2.1.2 Hypothesis 2: Genetic or obstetrical, rather than behavioral factors, contributed to the reduced production of offspring by grayfoot male/kinda female matings.

Background: If matings in the ancestral group were not significantly biased by ancestry, but some matings, like those between grayfoot males and kinda females, consistently failed to produce offspring, this asymmetry may have resulted from genetic or obstetrical, rather than behavioral factors. Kindas and grayfoots are highly disparate with regard to size, and thus a small kinda female might have difficulty carrying or successfully delivering a fetus fathered by a large grayfoot male. Alternatively, the possibility of genetic incompatibilities between kindas and grayfoots may have resulted in the miscarriage of fetuses or the failure of infants to survive into the postnatal period.

While we were unable to record the obstetrically relevant morphometric measures in the field, I was able to approach the question of genetic incompatibilities. Comparisons of PHIS correlations between the known mothers and fathers of infants with the GHIS correlations of known mother-father pairs can indicate the relative importance of the selected phenotypic features in mating that results in parenthood (i.e. premating mechanisms) compared to genetic features. Low parental PHIS correlations would

indicate lack of premating behavioral mechanisms, while high parental GHIS correlations would reflect the operation of some selective genetic mechanism, such that only genetically similar females and males produced offspring in the this group.

Prediction 13: If successful reproduction is a function of phenotypic similarity, mother-father PHIS correlations will be high and significant.

Prediction 14: If successful reproduction is an outcome of parental genetic similarity (or a lack of obstetrical incompatibility), then mother-father GHIS correlations will be high and significant.

2.1.3 Hypothesis 3: Grayfoot males misinterpreted kinda baboons as immature due to their small size, prompting inappropriate responses in the context of consortship and mating.

Background: When hybridization first occurred, grayfoot males may have misinterpreted kinda baboons as immature due to their small size, prompting inappropriate responses in the context of consortship and mating. It may be that grayfoot males did not attempt to deter matings by small, short-faced, "lanky" adult male kinda baboons who appeared to be juveniles rather than rivals. Also, since grayfoot males are attracted to females with larger sexual swellings, and are sexually uninterested in juvenile

females who tend to have smaller swellings [Zinner et al., 2002; Nitsch et al., 2011], it may be that grayfoot males misidentified the small simple sexual swellings of adult female kinda baboons to be the swellings of subadult females. Kinda males, on the other hand, may have interpreted the seemingly larger grayfoot female estrous swellings as supernormal, hyperattractive stimuli, and thus preferred to mate with them. Any of these behaviors could have contributed to the observed asymmetry.

For the time being, this hypothesis is offered for the sake of completeness, but the data at hand do not allow further investigation. It is worthy of testing in the future when there are adequate data on age of animals and scoring of estrous swellings.

In the work that follows, Chapter 3 describes the study site and subjects and outlines the methods used to create the male and female Phenotypic Hybrid Indices (PHI). Chapters 4 and 5 describe the genetic methods and data analyses used to create the Genetic Hybrid Index (GHI) and to assess the paternity of infants born before or during the study. Chapter 6 describes the protocols used to collect the behavioral data and the ways in which behavioral data were analyzed. Appendix 3 (page 230) describes the software (*Prim8 Mobile*) I created for this purpose. Chapter 7 provides the genetic, phenotypic, and behavioral results that address each prediction in Chapter 2. Chapter 8 discusses the results of the predictions in the context of each hypothesis. Chapter 9 summarizes the primary findings of this study and addresses how they contribute to hybrid zone theory and our knowledge of other baboon hybrid zones.

Chapter 3: Study Site and Study Animals

3.1 Study Site

3.1.1 Kafue National Park (KNP) and Game Management Areas (GMA)

Established in 1950, Kafue National Park (KNP) is the oldest and largest national park in Zambia and the second largest in Africa, covering approximately 22,400 km^2. It provides sanctuary for approximately 150 mammal species, 70 reptile species, 58 fish species, and 515 bird species [Zambia Wildlife Authority, 2010]. Nine Game Management Areas (GMAs) surround the majority of Kafue National Park and cover another 45,406 km^2 of land [Zambia Wildlife Authority, 2010] (Figure 6). GMAs are protected areas that act as buffer zones around the national parks and are areas in which regulated hunting is generally permitted.

KNP has a humid sub-tropical climate with the mean annual rainfall varying from approximately 700 mm in the south to 1100 mm in the north [Zambia Wildlife Authority, 2010]. The year can be divided into two main seasons: a rainy season (December – April) and a dry season (May – November). The dry season can be further divided into the cold dry windy season (May – August) and the hot dry season (September – November).

Figure 6: Map of Kafue National Park and Game Management Areas (GMAs); star indicates Ngoma, the study site, and Nkala GMA is the GMA where my group resided when outside of the national park.

Woodland (62%), grassland (19%), shrub land (14%), and forest (3%) are the primary vegetation types found in KNP. The woodland is primarily miombo woodland, a semi-deciduous and partially fire resistant woodland mainly composed of the three tree genera *Brachystegia*, *Julbernardia* and *Isoberlinia*. These woodlands are interspersed with open plains, some of which are seasonally flooded areas that are called "dambos". These dambos often hold water well into the dry months, keeping the grass lush for wildlife (www.zambiatourism.com/destinations/nationalparks/kafue-national-park). The above vegetation types can further be divided into the nine categories in Table 1, the first four can be found

throughout the park, the next two can be found centrally and the latter three are primarily in the southern part of the park.

Table 1: Vegetation types in Kafue National Park as described in ZAWA [2010]

Vegetation Type	Location within the Park	Description
Miombo Woodland	Throughout	This vegetation type is widely distributed throughout much of the Park. *Julbernardia paniculata* and *Brachystegia spiciformis* constitute the most dominant tree species in the canopy layer (8-15 m height). Other common tree species include *Diospyros batocana, Burkea africana, Erythrophleum africanum, Brachystegia boehmii, Pseudolachnostylis maprounefolia* and *Diplorhynchus condylocarpon*. The lower layer (2-6 m height) is characterized by sparsely distributed small trees and shrubs including *Bauhinia petersiana, Dalbegiella nyasae, Uapaca* species and *Diplorhynchus condylocarpon*. The structure shows local variation in height and coverage due to soil types, climatic conditions and fire occurrence.
Kalahari Woodland	Throughout	A vegetation type that has a similar structure to Miombo woodland and is widely distributed in the Park. Unlike the Miombo woodland, it has a characteristic discontinuous canopy cover. The canopy layer is formed around 12-16 m while the shrub layer height range is 2-4 m. *Julbernardia paniculata* constitutes the most dominant species followed by *Burkea africana, Parinari curatellifolia* and *Julbernardia globiflora*. The shrub layer is dominated by *Terminalia mollis, Diospyros batocana* and *Pseudolachnostylis maprounefolia*.
Termitaria Vegetation	Throughout	Termitaria vegetation is characterised by plant communities that are established on large termite mounds. Three types have been recognised: Mopane, Riparian and Munga. The best example of Mopane Termitaria is found in the Nanzhila Plains and Riparian Termitaria along the Lufupa River to Busanga Plain. This brief description relates to the Mungatermitaria because the other two types have not been surveyed in detail. *Diospyros mespiliformis, Euphorbia ingens* and *Capparis tomentosa* constitute the dominant species in the canopy layer (14-16 m height).

		Below the canopy layer, *Garcinia livingstonei* is dominant while the small trees and shrubs are dominated by *Albizia anthelmintica, Sapium ellipticum* and *Ximenia americana*. The climber, *Fockea multiflora* is common.
Grassland	Throughout	Grasslands may be either derived in places where fire and heavy browsing occur or are edaphic, as in dambos and floodplains. Dambo grasslands are found throughout the Park and constitute the major grassland community. Riverine grasslands are confined to alluvial soils along riverbanks. Floodplain grasslands are mainly found on Busanga and Nanzhila Plains while smaller areas are found along the Lufupa, Lwansanza and Musa Rivers. The dominant species belong to the genera of *Loudetia, Monocymbium, Eragrostis, Setaria, Digitaria* and *Themeda*.
Riparian Woodland	Central	This evergreen vegetation type is characterized by continuous or discreet strips of dense shrub layer (2-8 m height) with few emergent trees reaching about 15 m and is well represented in the Park and is found on riverbanks and islands along the Lufupa and Kafue Rivers. *Diospyros mespiliformis, Sapiumn ellipticum* and *Syzygium guineense* constitute the common tree species. The shrub layer is dominated by *Azanza kirkiana, Oncoba spinosa, Homalium abdessammadii, Maytenus buchmannii* and *Securinega virosa*.
Munga Shrubland	Central	This mainly derived vegetation type is characterized by the dominance of *Bauhinia thonningii, Acacia nilotica* and *A. polyacantha* and comparatively low species diversity. The spatial structure is sparse with the tree height range of 2-6 m. Associated shrub species are *Combretum fragrans* and *Turrae nilotica*.
Baikiaea Forest	South	This deciduous vegetation type is dominated by *Baikiaea plurijuga* and *Pterocarpus antunesii* with a canopy layer in the range 15-20 m. *Friesodielsia obovata* and *Pterocarpus antunesii* dominate the shrub layer in the height range of 2-5 m. *Baphia massaiensis* shrubs grow in dense clusters immediately adjacent to some portions of the forest. This vegetation type is restricted to well-drained Zambezi deep sands in

		the southern part of the Park. Ngoma Forest is the best example of this vegetation that was probably much more widely distributed in the past.
Secondary Baikiaea Woodland	South	Degraded Baikiaea Forest. Although *Baikiaea plurijuga* and *Pterocarpus antunesii* are usually dominant, *Combretum collinum* and *Xeroderris stulmannii* are by far the most common in the canopy layer (12-16 m height). This vegetation type is characterized by moderate coverage of canopy layer and dense shrub layer (2-6 m height). The shrub layer is dominated by *Friesodielsia obovata* and *Markhamia obtusifolia* while *Pseudolachnostylis maprounefolia* and *Combretum collinurn* are also common.
Mopane Woodland	South	This vegetation type is characterized by almost pure stands of *Colophospermum mopane*. The occasional associated tall tree species include *Adansonia digitata* and *Acacia nigrescens*. Common species in the shrub layer include *Boscia matabelensis, Markhamia acuminata, Sclerocarya birrea* and *Balanites aegyptiaca*. The lower layer is dominated by *Securinega virosa, Duranta ripens* and *Strychnos usambarensis*.

The Park can more specifically be broken down into three geomorphological zones: North, Central and South. North KNP is characterized by the Busanga Floodplains overlying "Kalahari" sand with the presence of papyrus swamps, hot springs, miombo woodlands on sandy soils, and an increased number of termitaria with related vegetation on cracking clay soil. Central and Southern KNP is characterized by miombo woodland vegetation combined with grassland on sand, as well as Teak forests and belts of Mopane woodland. Mopane woodland refers to woodland primarily consisting of *Colophospermum mopane* trees, trees with distinctive butterfly-shaped (bifoliate) leaves and thin seed pods [Van Wyk & Van Wyk, 1997]. The Teak and Mopane are more prevalent in the southern part of the Park and light brown to "red leached

plateau soils", pale grey clays, and termitaria vegetation is more prevalent in Central KNP [Zambia Wildlife Authority, 2010].

The primary river in Kafue National Park is the park's namesake, the Kafue River, which is the largest tributary of the Zambezi River. This river feeds into Lake Itezhi-tezhi (370 km^2) as well as the Lufupa and Lunga Rivers.

3.1.2 Ngoma, the Southern Headquarters of Kafue National Park and Nkala GMA

This research project was carried out at the Park's Southern Headquarters in Ngoma (15.96495S, 25.93955E). This site is located in Kafue National Park, approximately 30 km from Itezhi-tezhi, near a portion of the neighboring Nkala GMA (Figure 6). The environment in this area is characterized by a rainy season from December through March and a dry season from April to November. In May and June prescribed burning is carried out in areas throughout the park and neighboring GMAs--aside from the protected Ngoma Teak Forest--in order to promote grass growth for the animals and to allow for better viewing of wildlife. The annual mean temperature around Ngoma ranges from 19.4ºC to 21.7ºC. The hottest month is October, with a mean maximum temperature range of 30.8ºC and 34.9ºC and a mean minimum temperature range of 14.9ºC to 17.6ºC. July is the coldest month, with maximum temperature ranges of 22.3ºC to 28.3C and minimum temperature ranges of 4.7ºC to 7.3ºC. Humidity in the area ranges from 34.3% in September to 78.1% in February [Zambia Wildlife Authority, 2010].

3.2 Study Group

While several baboon groups in the area contained hybrid individuals, the GMA group was chosen as the focal group as it was the most convenient for our trapping study (page 33) due to our prior familiarity with the area and the geographical proximity of these baboons to Ngoma, the southern headquarters of the Kafue National Park (15.96S, 25.93E) (Figure 5), This section describes the trapping and radio collaring procedure, the habituation process and the methods used in creating the phenotypic hybrid index. It ends with photos of all adult males and females and some general information regarding the reproductive status of females within the group.

3.2.1 Trapping & Radio Collaring

From May-June 2012, as part of an ongoing baboon trapping study undertaken by Phillips-Conroy and Jolly's research group, I assisted in trapping baboons from this phenotypically admixed focal group. We used the trapping procedure from Jolly et al. [2011], whereby we habituated animals to traps, baited them with maize, and used a pole dart with Ketamine hydrochloride (10 mg/kg body weight) to anesthetize the animals. While anesthetized, we recorded body mass, obtained dental casts, and collected several biological samples for future studies, including blood, feces, and hair. To facilitate the identification, tracking, and habituation of this group for my research and behavioral data collection, we radio collared (ATS, Isanti, MN) two individuals: an adult female, Selala, and an adult male, Big K. We had intended to radio collar two females, as males are expected to migrate; however, we were only able to trap the one female. We used two 1.5 inch 5-ply neoprene/3-ply pocket baboon collars (M2230B; 190 grams,

warranty life 1095 days, battery life 3796 days) that were fitted with SureDrop Breakoff Mechanisms (SD30), a 15 inch 1/8 OD Black antenna and a mortality signal option. I then tracked these animals using a R410 scanning receiver and a3-Element Folding Yagi antenna.

3.2.2 Habituation and Identification

Once the trapping study ended, I spent the next five months habituating the group to my presence so behavioral follows could be recorded (see Chapter 6 on page 125). This kinda x grayfoot hybrid group consisted of 64 individuals: 14 adult males and 20 adult females (all which were individually identifiable), and 30 subadults, juveniles and infants. While I initially expected the baboons to be permanently resident in Kafue National Park, it soon became clear that they frequently crossed the park boundary and primarily resided in the Nkala Game Management Area (GMA) adjacent to the Park (Figure 6). Since July through September was a prime hunting season in the GMA, I was often forbidden from following the group, as it was thought that doing so would interfere with the hunting and would put me in danger. Despite these interruptions, by mid-December (2012), all of the adult males and most of the adult females in the group had been identified and were habituated by my research team, which included my field assistant, Isaac, and a rotating scout. Although limited baiting and trapping have repeatedly been shown to have little or no lasting effect on behavioral data collection [Nystrom, 1992; Beehner, 2003], there is always some concern that trapping could alter baboon behavior in some way. Thus, I allowed for a lengthy, 5-month habituation process before collecting systematic behavioral data. This also provided me with sufficient time to optimize behavioral data collection protocols, fine-tune the electronic data collection software (*Prim8 Mobile*) and device (page

230), train Isaac in behavioral data collection methods, and collect fecal samples from all identified individuals for genetic analyses.

I used a combination of unique physical features to identify each animal, including body size and shape, pelage, tail length and shape, ear and browridge shape, the size, shape, and clarity of white facial patches, and other imperfections, such as scars, tears, or punctures. I photographed the face (front and profile) and body (side profile) of each animal and made identification cards to aid in correctly identifying individuals (Table 5 and Table 6).

During the period of data collection (described in Chapter 6), the focal study group (GMA group) spent most of the observation time foraging (52% of 734 hours). During the dry/burning season (as mentioned below), they were occasionally observed to join together with another neighboring group ("the Shy Group"), primarily fusing in the evenings at their sleeping sites and then fissioning the following morning. When we managed to arrive in the morning before the combined group was awake, the individuals would calmly come down from the trees and the study group would go one direction and the "Shy Group" would go another. On other occasions, when we found the combined group *after* they were already foraging, the entire group would run for a short distance, at which point, the unhabituated "Shy Group" would depart, while the habituated group would remain.

3.3 Phenotypic Classification

I scored each individual's phenotypic characteristics (Table 2) based on observations and photographs of each individual as follows: Kinda-like (0), Intermediate (1) or Grayfoot-like (2)

(Table 5 and Table 6). Male and female hybrid phenotypes were measured based upon the phenotypic extremes of individuals seen in this group. Females were additionally scored for sexual swellings when in estrus (Table 4). These scores were then summed across characteristics to calculate a phenotypic hybrid index for each animal [Nagel, 1973; Bergman, 2000; Alberts & Altmann, 2001; Jolly et al., 2010] (Table 3 and Table 4). Every animal was scored by myself and Isaac. As all baboons could not be scored for all three scoring sessions, some individuals were only scored once or twice. These three scoring sessions were averaged per individual, and females were given two types of scores based on whether they were observed in estrus or not (Table 4). These average scores were then normalized to give the Phenotypic Hybrid Index Score (PHIS) using the following formula: $(x - x_{min})/(x_{max}-x_{min})$, where x is the average score for the individual of interest and x_{min} and x_{max} are the lowest and highest average raw scores across all individuals, respectively. PHIS were then used to allocate individuals into 3 categories: Kinda-like, Grayfoot-like, and Intermediate (Figure 7 and Figure 8). The Kinda-like, Intermediate, and Grayfoot-like categories were based upon natural breaks in the normalized scores after visualizing both the normalized scores and the differences between them (Figure 7 and Figure 8). The group consisted of 7 Kinda-like, 6 Grayfoot-like, and 3 Intermediate males and 5 Kinda-like, 11 Grayfoot-like, and 4 Intermediate females. Among the males, an individual named Uno represented the kinda extreme (0) and Kuyipa represented the grayfoot extreme (1); among the females, Yang represented the kinda extreme (0) and Helena represented the grayfoot extreme (1). See Table 5 and Table 6 for individual animal photos and hybrid scores. Details of the genotypic assessments are fully described in Chapter 5 (page 117). **For analyses using phenotypic categories, the individuals will be coded as follows: Kinda-like = Kp, Intermediate = Ip, and Grayfoot-like = Gp**.

Table 2: Characteristics used to calculate the Phenotypic Hybrid Index Score

Character	Kinda (0)	Intermediate (1)	Grayfoot (2)
Light Muzzle Hair Patches	Absent	Slight	Present, conspicuous
Nape Hair (males only)	Not prominent; yellow	Visible frill small (intermediate color)	Long, curled, black
Punk Crest (mohawk)	Marked mohawk (crown hair)	Some	None; even length or slight median crest
Circumorbital Skin	Light pink eye "spectacles"	Intermediate	Dark (except pregnant female)
Tail Carriage	Usually high arch; "riding whip"	Broken, proximal part is horizontal	Usually "broken" with flatter arch
Relative Tail Length	Shorter, reaches knee	Intermediate, just below knee	Longer, reaches calves
Dorsal /Ventral Hair Color	Yellow-brown/little to no contrast	Intermediate	Drab gray-brown/ light gray
Body Build	Small, gracile, long limbs	Intermediate	Large, robust appearance
Sexual Swelling (estrous females only)	Small heart-shaped	Intermediate	Big inflated balloon

Table 3: Male Composite PHIS and Assigned Phenotypic Category

Adult Males	Asst Score	MM Score 1	MM Score 2	Avg. Score	Normalized Score	Phenotype Assignment*
Big K	7	8	8	7.67	0.15	Kinda-like
Chifupi	10	8	7.5	8.50	0.24	Kinda-like
Duo	-	10	5	7.50	0.13	Kinda-like
Jack	14	15	13	14.00	0.84	Grayfoot-like
Kink	8	7	5	6.67	0.04	Kinda-like
Kuyipa	16	15	15.5	15.50	1.00	Grayfoot-like
Mavuto	16	15	13.5	14.83	0.93	Grayfoot-like
Old Kuyipa	-	-	14	14.00	0.84	Grayfoot-like
R. Simmons	12	11	10.5	11.17	0.53	Intermediate
Solomon	14	-	16	15.00	0.95	Grayfoot-like
Spock	16	12	14	14.00	0.84	Grayfoot-like
Strider	11	8	9	9.33	0.33	Intermediate
Uno	7	6	6	6.33	0.00	Kinda-like
Subadults	9	9	6	8.00	0.18	Kinda-like
Valentino	8	5	6	6.33	0.00	Kinda-like
Yogi	10	8	10.5	9.50	0.35	Intermediate

*Phenotypic breakdown based upon normalized scores: Kinda-like =<0.25, Mixed=0.25-0.75, Grayfoot-like=>0.75

Asst Score, MM Score 1, and MM Score 2 = the 3 scoring sessions mentioned above

Table 4: Female Composite PHIS and Assigned Phenotypic Category

Adult Females	Asst Score	MM Score 1	MM Score 2	Avg. Score 1*	Avg. Score 2**	Final Avg. Score	Normalized Score	Phenotype Assignment***
April	11	-	9	10.50	10.00	10.50	0.59	Intermediate
Arwen	8	5	7	**NA**	6.67	**6.67**	0.24	Kinda-like
Chilonda	14	-	13	**NA**	13.50	**13.50**	0.86	Grayfoot-like
Daisy	14	10	11	12.00	11.67	12.00	0.73	Grayfoot-like
Flora	8	10	14	**NA**	10.67	**10.67**	0.61	Intermediate
Gaga	12	-	9	**NA**	10.50	**10.50**	0.59	Intermediate
Helena	16	-	14	**NA**	15.00	**15.00**	1.00	Grayfoot-like
Jean	8	8	12	10.50	9.33	10.50	0.59	Intermediate
Jesmine	9	-	10	9.50	9.50	9.50	0.50	Kinda-like
Merry	12	-	10	12.50	11.00	12.50	0.77	Grayfoot-like
Minnie	13	10	17	13.50	13.33	13.50	0.86	Grayfoot-like
Ndona	16	10	13	14.00	13.00	14.00	0.91	Grayfoot-like
Ophilia	13	-	11	12.50	12.00	12.50	0.77	Grayfoot-like
Queenie	16	-	13.5	**NA**	14.75	**14.75**	0.98	Grayfoot-like
Rosy	6	7	10	9.50	8.67	9.50	0.50	Kinda-like
Selala	12	10	13	13.50	11.67	13.50	0.86	Grayfoot-like
Tamanga	-	10	13.5	**NA**	13.25	**13.25**	0.84	Grayfoot-like
Yang	4	4	4	**NA**	4.00	**4.00**	0.00	Kinda-like
Yin	6	8	7	9.00	7.00	9.00	0.45	Kinda-like
Zelda	14	11	13	**NA**	12.67	**12.67**	0.79	Grayfoot-like

*average score with swelling size and shape included in index

**average score without swelling size and shape included in index

***Phenotypic categories based upon normalized scores: Kinda-like=<0.50, Mixed=0.51-0.69, Grayfoot-like=>0.70)

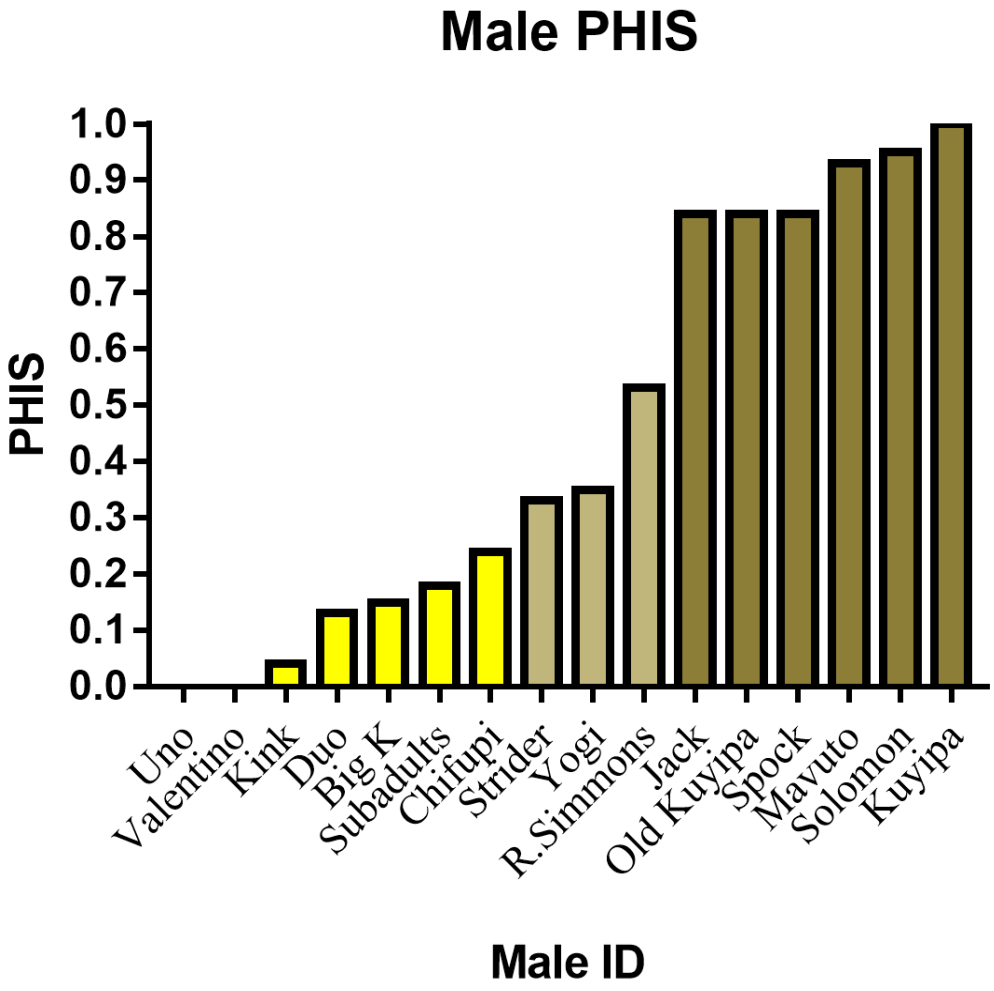


Figure 7: Male PHIS and PHIS Categories; Kinda-like (yellow; n=7), Intermediate (light brown; n=3), and Grayfoot-like (dark brown; n=6) categories were created using the natural breaks in the ordered scores.

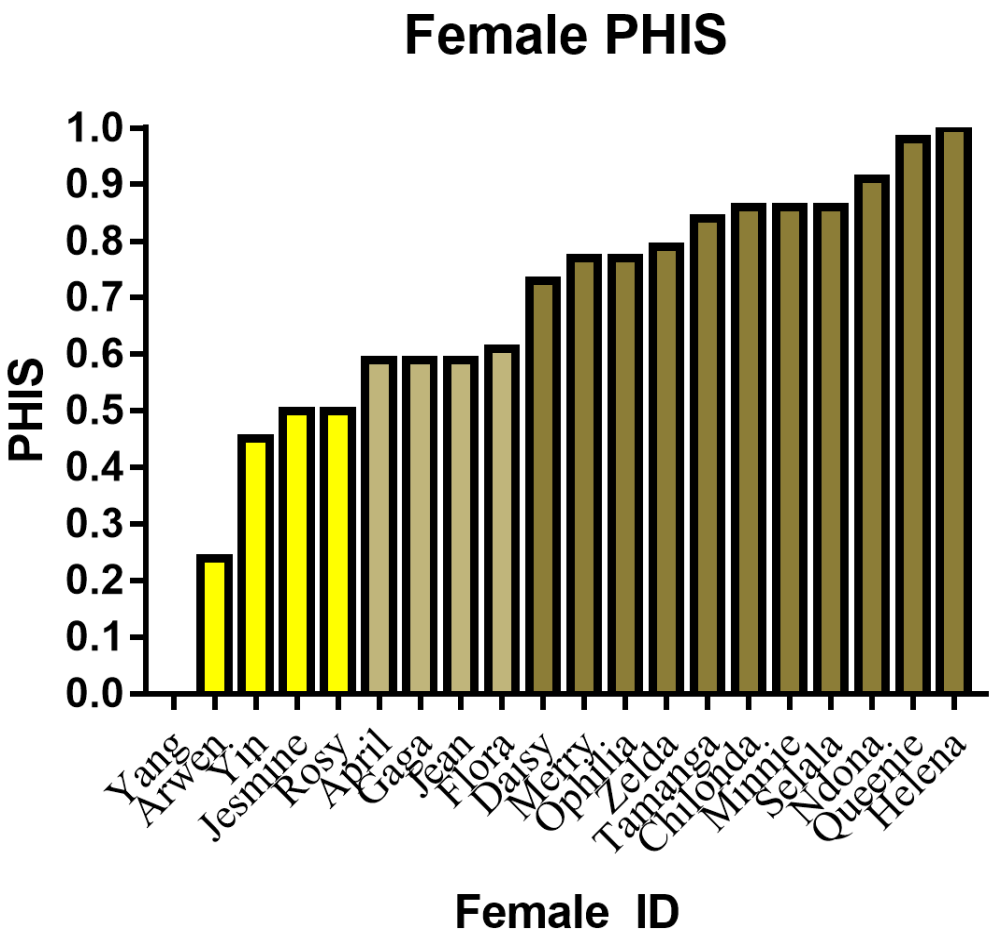


Figure 8: Female PHIS and PHIS Categories; Kinda-like (yellow; n=5), Intermediate (light brown; n=4), and Grayfoot-like (dark brown; n=11) categories were created using the natural breaks in the ordered scores.

3.3.1 Identified Study Group Individuals

Table 5 and Table 6 below show each identified group member, along with his or her phenotypic and genotypic hybrid index score (PHIS and GHIS). Details describing the phenotypic characteristics used to arrive at their PHIS were described previously (page 35) and those describing details regarding the microsatellites and methods used to arrive at their GHIS can be found in Chapter 4 (page 97) and Chapter 5 (page 117).

Table 5: Adult Male Information: males are shown alphabetically by name and data entry code; PHIS are individual scores for each individual's phenotype (based upon 8 physical characteristics) (0-1 scale); GHIS are individual scores for each individual's genotype (based upon 7 microsatellite loci) (0-1 scale); 0 = Kinda; 1 = Grayfoot.

Big K (Bk) PHIS: 0.15; **GHIS:** 0.57

Chifupi (Ch) PHIS: 0.24; **GHIS:** 0.38

Jack (Jk) PHIS: 0.84; **GHIS:** 0.06

Kink (Ki) PHIS: 0.04; **GHIS:** 0.93

Old Kuyipa PHIS: 0.84; **GHIS:** 0.86

Kuyipa (Ku) PHIS: 1.00; **GHIS:** NA (unable to be genotyped)

Mavuto (Ma) PHIS: 0.93; **GHIS:** 0.14

Richard Simmons (RS) PHIS: 0.53; **GHIS:** 0.98

Solomon (So) PHIS: 0.95; **GHIS:** 0.02

Spock (Sp) PHIS: 0.84; **GHIS:** 0.88

Strider (St) PHIS: 0.33; **GHIS:** 0.03

Uno (Un) PHIS: 0.00; **GHIS:** 0.31

Valentino (Va) PHIS: 0.00; **GHIS:** 0.74

Yogi (Yo) PHIS: 0.35; **GHIS:** 0.12

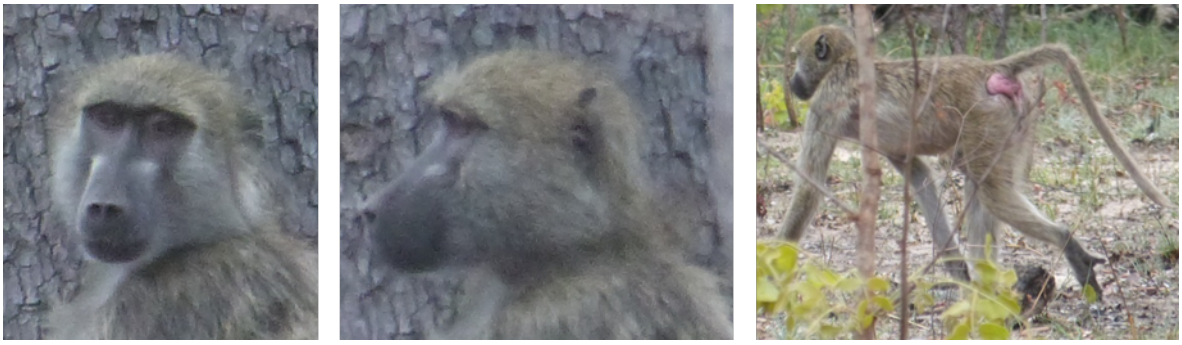
Subadult Males (Cm & Dm) PHIS: 0.18 (scored together); **GHIS:** 0.05 (only one scored)

Table 6: Adult Female Information: females are shown alphabetically by name and data entry code; PHIS are individual scores for each individual's phenotype (based upon 8 physical characteristics) (0-1 scale); GHIS are individual scores for each individual's genotype (based upon 7 microsatellite loci) (0-1 scale); 0 = Kinda; 1 = Grayfoot.

Arwen (Ar) PHIS: 0.24; **GHIS:** 0.57

April (Al) PHIS: 0.59; **GHIS:** 0.11

Chilonda (Cl) PHIS: 0.86; **GHIS:** 0.02



Daisy (Da) PHIS: 0.73; **GHIS:** 0.05

Flora (Fl) PHIS: 0.61; **GHIS:** 0.08

Gaga (Ga) PHIS:0.59; **GHIS:** 0.07



Helena (He) PHIS: 1.00; **GHIS:** 0.43



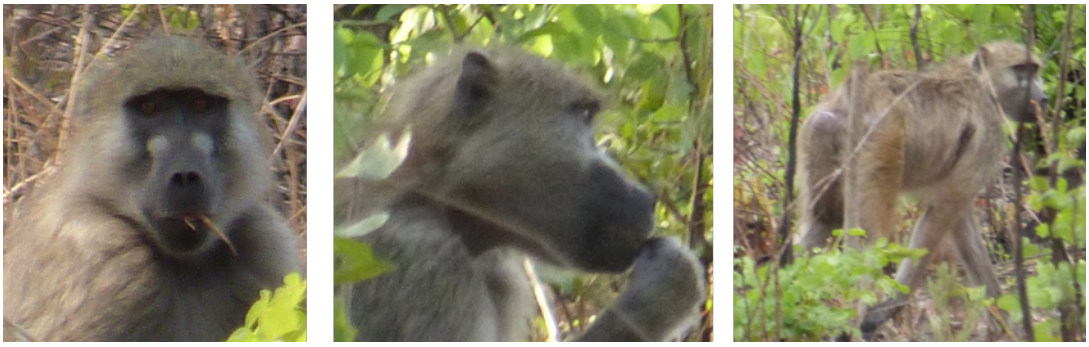
Jean (Je) PHIS: 0.59; **GHIS:** 0.20



Jesmine (Jm) PHIS: 0.50; **GHIS:** NA (unable to be genotyped)

Merry (Me) PHIS: 0.77; **GHIS:** NA (unable to be genotyped)

Minnie (Mi) PHIS: 0.86; **GHIS:** 0.20



Ndona (Nd) PHIS: 0.91; **GHIS:** 0.31

Ophilia (Op) PHIS: 0.77; **GHIS:** 0.27

Queenie (Qu) PHIS: 0.98; **GHIS:** NA (recollected sample in 2015; found to be same genotype as Zelda)

Rosy (Ro) PHIS: 0.50; **GHIS:** 0.09

Selala (Se) PHIS: 0.86; **GHIS:** 0.23

Tamanga (Ta) PHIS: 0.84; **GHIS:** NA (unable to be genotyped)

Yang (Ya) PHIS: 0.00; **GHIS:** 0.57

Yin (Yi) PHIS: 0.45; **GHIS:** 0.47

Zelda (Ze) PHIS: 0.79; **GHIS:** 0.84 (found to be same genotype as Queenie)

3.3.2 Female Reproductive Condition

I evaluated all adult females in this group daily for their basic reproductive condition, categorizing each as estrous, pregnant, or lactating [Nystrom, 1992; Beehner, 2003]. I further specified each cycling female's reproductive state as flat, menstruating, early-estrus, mid-estrus, peak estrus, deflating, or deflated following Beehner [2003] (Table 7). When possible, I photographed the shape and color of the peak estrus swelling [Higham et al., 2008; Huchard et al., 2009] The onset of detumescence (rapid deflating of the swelling) is known as D-day or D-0,

and an estrus cycle is defined as the time between d-days (~28 days) [Nystrom, 1992; Altmann & Alberts, 2003]. Ovulation occurs 1-3 days prior to detumescence (D-3 to D-1) [Bulger & Hamilton, 1988; Altmann & Alberts, 2003; Moscovice et al., 2009] and is strongly coincident with maximal tumescence (peak swelling size), although female baboons have been known to conceive up to 7 days prior to ovulation, when their swelling is only partially inflated. Gestation length is approximately 6 months. While early stages of pregnancy are marked only by cycle cessation, later pregnancy is accompanied by marked changes in perineal skin color, from grey to bright pink in the last trimester. I noted these changes and calculated first, second, and third trimester pregnancy stages (2 months each) retrospectively from the birth of the infant [Altmann, 1973; Beyene, 1998; Higham et al., 2008; Huchard et al., 2009]. I assigned developmental stages based upon the coloration and development of the infant [Beyene, 1998] (Table 7). When possible, photographs of each female were taken during various states of estrus, pregnancy, and infant development for long-term use and to help further document female reproductive condition.

Table 7: Female Reproductive Condition

Code	Reproductive Stage	Definition
M	Menstruation	menstrual blood present on perineal skin
E1	Beginning of estrus	slight turgescence of perineal skin
E2	Mid-estrus	inflating turgescence of perineal skin
E3	Peak estrus	peak turgescence of perineal skin
E4	Deflating	detumescence of perineal skin (d-day)
E5	Deflated	deflated perineal skin
F	Flat	flat perineal skin
P1	First trimester	months 1-2 of pregnancy
P2	Second trimester	months 3-4 of pregnancy
P3	Third trimester	months 5-6 of pregnancy
L1B	Black Infant	early lactation/development
L1W	White Infant	early lactation/development
L2	Transitional infant	mid lactation/development
L3	Brown infant/weaning	late lactation/development

Figure 9 and Figure 10 shows the reproductive condition of each female throughout the study. Two of the 20 females already had infants when the observation period began, eight females in the group gave birth during the observational period, and three females gave birth shortly thereafter (Figure 9). During the observational period and during the summer of 2014, I was able to opportunistically collect and genotype samples from 10 infants and another three unknown juveniles. These samples were typically collected when multiple infants were playing together so maternal identity was not certain.

One female, Tamanga, and her infant disappeared from the group in February 2013. Aside from this female, between February and July 2013, I recorded behavioral data for all adult females. During this time, eight females underwent estrus cycles. I was able to record mating behavior across three estrus cycles for three females (Yin, Ophilia, April), across two estrous

cycles for three females (Ndona, Merry, Jesmine), and throughout one estrous cycle for two females (Rosy, Jean) (Figure 10 and Table 8).

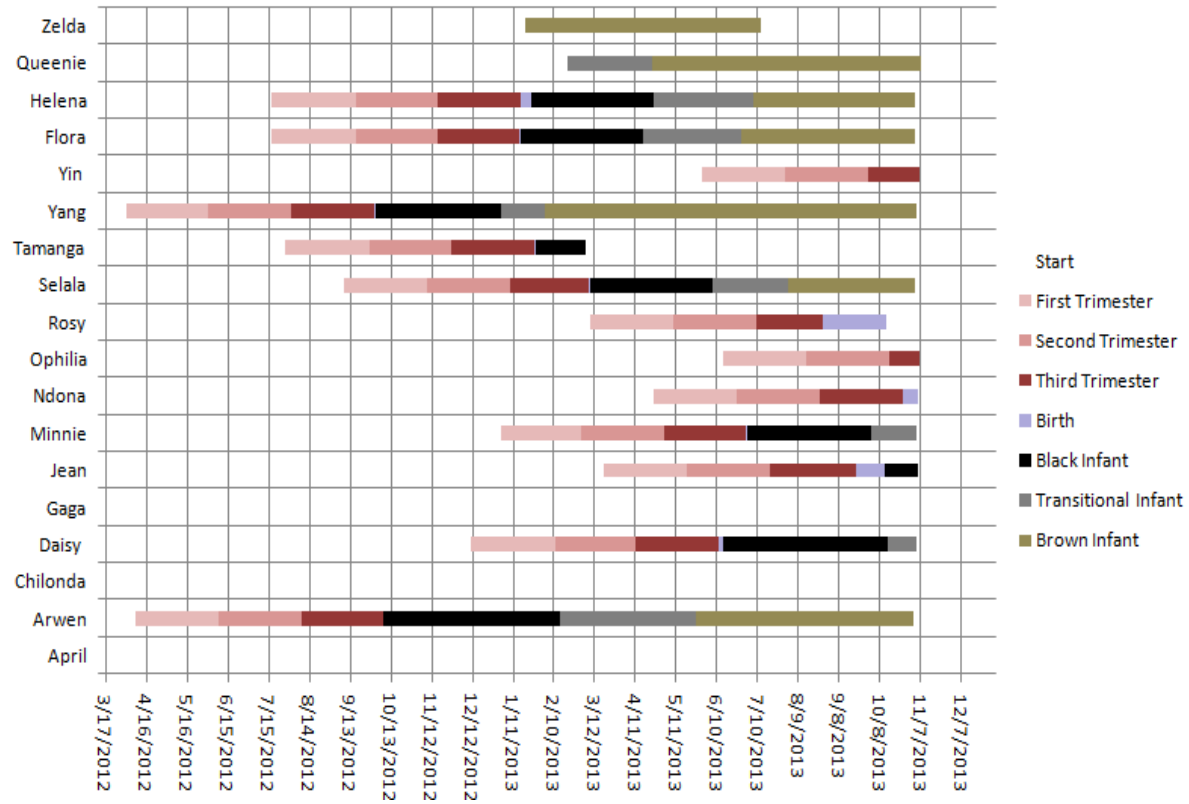


Figure 9: Pregnancy and lactation timeline; see Appendix 4 for more details

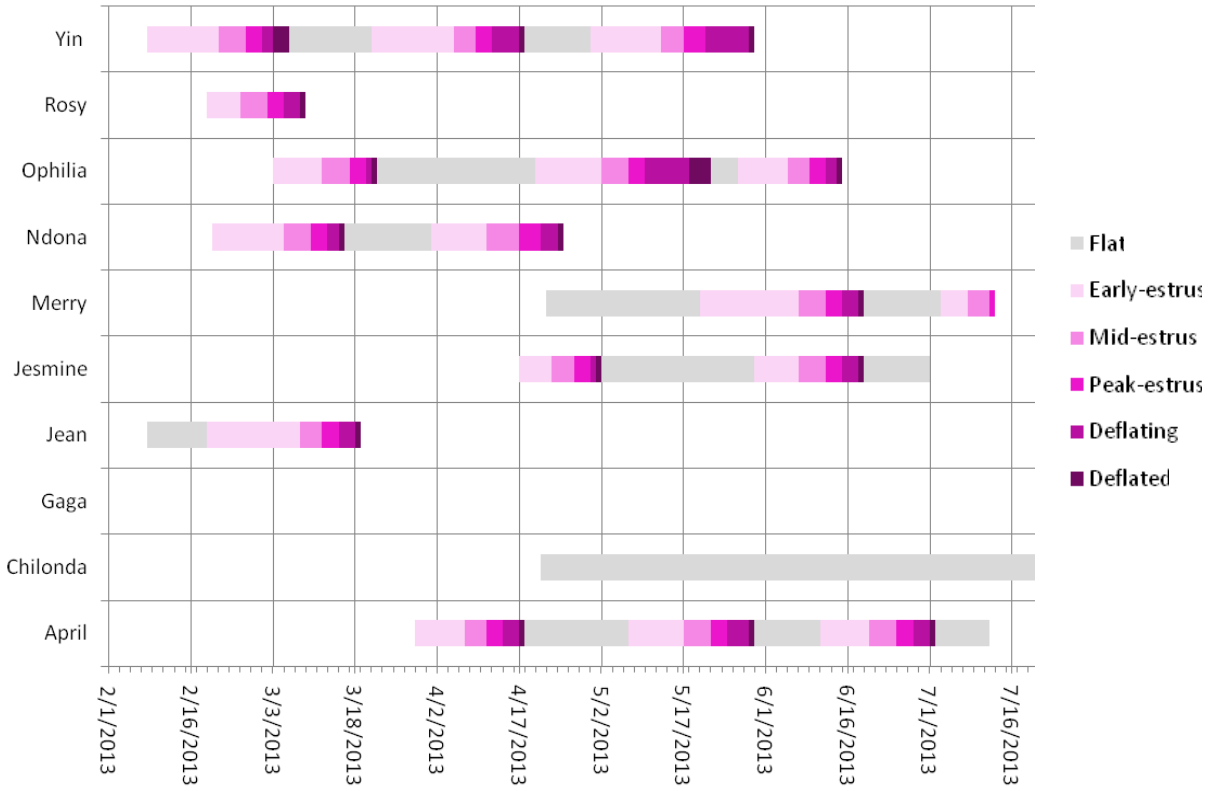


Figure 10: Estrus timeline; estrus state for each female that was not pregnant or lactating. Gaga was not yet cycling and Chilonda was an old female.

Table 8: Number of cycles, pregnancies, and offspring by each adult female during the study period.

Female ID	Estrous Cycles (2012-2013)	Pregnant** (2012-2013)	# of Offspring (2012-2013)
April[1]	3	0	1
Arwen	0	1	1
Chilonda	0	0	0
Daisy	0	1	1
Flora[1]	0	1	2
Gaga	0	0	0
Helena	0	1	1
Jean[2]	1	1	1
Jesmine	2	0	0
Merry	2	0	0
Minnie	0	1	1
Ndona	2	1	1
Ophilia	3	1	0*
Rosy	1	1	0*
Selala	0	1	1
Tamanga	0	1	1
Yang	0	1	1
Yin	3	1	0
Zelda[2]	0	0	1

[1]Females that had one offspring in 2014 or 2015 from whom parentage was obtained
[2]Female that gave birth to white baby in 2016 (no genetic data obtained)
** Indicates females that went from being in their third trimester to no longer being pregnant, but with no infant*
***1 = pregnant and 0 = not pregnant*

3.4 Study Group Ranging Area

This hybrid group had a home range of 92 km^2 and a perimeter of 39 km (Figure 11). During the study period, the group spent most of its time approximately 4 to 14km south of Ngoma and ranged up to 10 km in an East/West direction. The areas in which they spent their

time each month can be seen in Figure 12 and Table 9. Their primary ranging area consisted of at least 10 major sleeping sites that shifted monthly and seasonally, including the trees surrounding the Ngoma Airstrip (approximately 7 km south of Ngoma). Their home range also included at least 10 major water points that were relied upon differentially depending upon the time of year. All but one of these dried up in the extreme of the dry season (November), leaving only a mud patch with underlying clay rather than sandy soil, where the baboons dug to find stagnant water. Ngoma is located on the eastern border of the Park so the baboon group ranges both within KNP and the neighboring Nkala GMA (Figure 11). This GMA is characterized by the same general environmental conditions; however, hunting of a variety of wildlife is allowed by permit in these areas. The baboon home range almost extended to the area outside of these protected areas and was only a few kilometers from several villages. Because their home range did not extend into these human-inhabited areas, these baboons were not pests and rarely came into contact with humans. The exception to this was when they ranged near the Ngoma Airstrip (Figure 11). The building at the airstrip is inhabited by 1-2 wildlife police officers approximately half of the year, particularly during tourist season in order to collect park fees from visitors flying into the airstrip. Even with their close proximity to humans, groups did not raid nearby areas for human food.

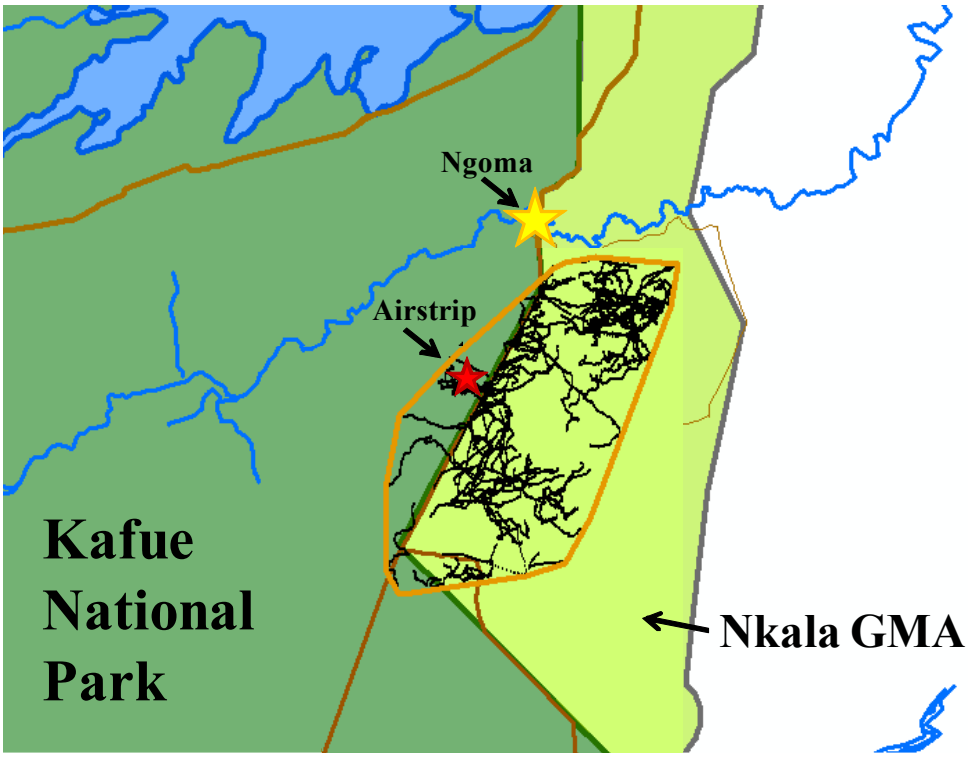


Figure 11: Map of study group's general ranging area near Ngoma; Ngoma is denoted with a yellow star and the Ngoma Airstrip is denoted with a red star. Kafue National Park is shown in dark green and the neighboring Nkala GMA is in light green. The polygon is the Minimum Convex Polygon that encompasses the group's home range (92 km^2). The lines within the polygon represent each daily track log (n=108) recorded during the observation period.

One other baboon group shared a part of the study group's home range and a third group was also occasionally observed. It was suspected that these groups had belonged to a single large group that had recently divided into 2-3 groups because at times, typically during the dry season/burning season, the two (and sometimes even three) groups could be found ranging and/or sharing the same sleeping trees (fusing) before splitting off into separate groups again (fissioning). Trees at the Ngoma airstrip were the primary sleeping site that they shared during this time, however, they were noted to occasionally share other sleeping sites as well. This

group-based fission-fusion pattern [van Schaik, 1999] has been occasionally observed since then--between May and July--in 2014 and 2015 by me and in 2016 by my field assistant, Isaac. This supports the idea that this is a regular fission-fusion pattern. This behavior has only been observed during the dry season, when many free-standing water holes have dried up, and during hunting season in the GMA. Thus, it is likely that this fission-fusion pattern may help guard against predators (including humans) at times when the baboons have longer day ranges due to reduced water availability in the area.

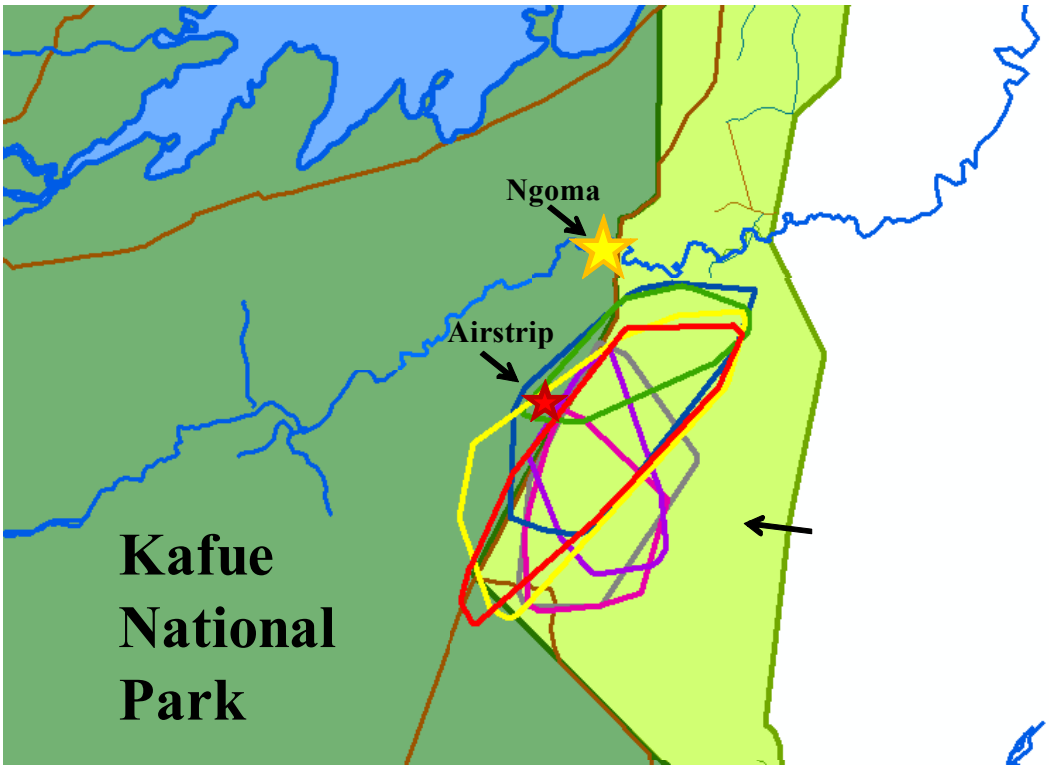


Figure 12: Baboon group's home ranges (100% MCP) by month; Red = January, Yellow = February, Green = March, Blue = April, Purple = May, Pink = June, Gray = July.

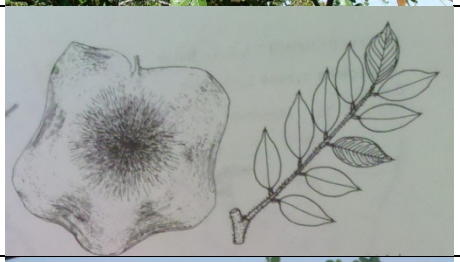
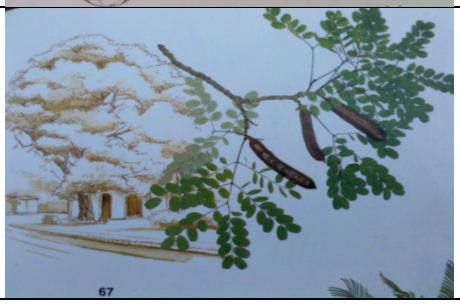
Table 9: Areas and perimeters of the Minimum Convex Polygons (MCPs) that represent the focal hybrid group's monthly and total home ranges. Total numbers represent the polygon created using all track logs, whereas monthly numbers represent polygons created using only the track logs from each month.

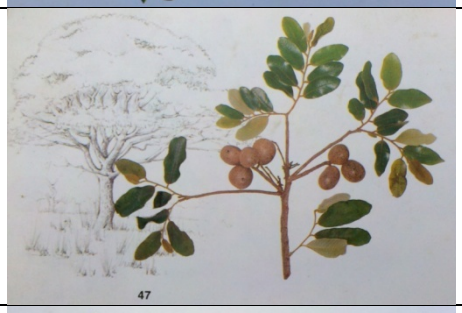
Month	Area (km^2)	Perimeter (km)
January	45.1	33.1
February	59.9	34.1
March	23.2	21.1
April	51.1	29.7
May	27.9	21.5
June	29.0	21.1
July	41.6	26.4
TOTAL	**92.8**	**39.1**

3.5 Study Group Diet

During the data collection period from February to July 2013, the group was observed to forage on many types of plants, the most common of which can be seen in Table 10. The group was also observed to feed on grasses and corms, as well as insects and small mammals. Specifically, the group killed and consumed 4 small duikers, 2 rabbits, and a bird during the 2012-2013 field season (Figure 13 and Figure 14).

Table 10: Plant species that this group was commonly found eating [van Wyk, 1997].

Scientific Name	Common Name	Common Food Sources
Brachystegia spiciformis	Msasa	
Julbernardia globiflora		
Isoberlinia angolensis		
Pterocarpus angolensis	Mukwa Bloodwood	
Amblygonocarpus andongensis	Scotsman's Rattle	

Gubourtia coleosperma	Large False Mopane	
Parinari curaellifolia	Mobola Plum	
Acacia erioloba	Camel Thorn	
Baikiaea plurijuga	Rhodesian Teak	
Strychnos cocculoides	Corky-bark Monkey Orange	

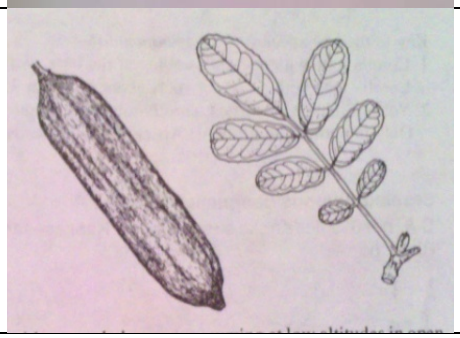
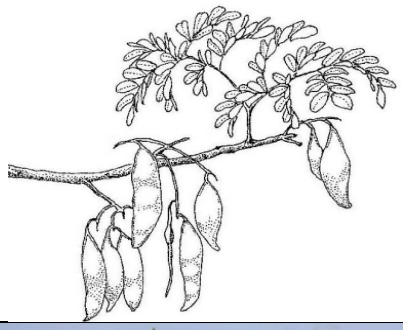
Diospyros mespiliformis	Ebony Diospyros	
Kigelia africana	Sausage Tree	
Erythrophleum africanum	Ordeal Tree	
Terminalia sericea	Silver Terminalia	

Figure 13: Baboon consuming small duiker after having just caught it.

Figure 14: Partially eaten rabbit that baboons were feeding on after having just caught it.

Chapter 4: Genetic Methods

4.1 Fecal Sample Collection and Preservation of Samples

Previous studies have demonstrated that DNA can be extracted, amplified and sequenced from mammal fecal samples [Jensen-Seaman & Kidd, 2001; Murphy et al., 2002; Clifford et al., 2004; Bergman et al., 2008; Jolly et al., 2010; Snyder-Mackler et al., 2012]. Between February 2013 and June 2015, I collected 243 baboon fecal samples for DNA analysis (185 in 2012-2013, 50 in 2014 and 8 in 2015). Fecal samples were collected when fresh to minimize DNA degradation and were preserved in a 2:1 ratio of *RNAlater* (Ambion, Austin, TX), a buffer that keeps DNA stable at room temperature for months [Burrell, 2009; McDonald & Hamilton, 2010]. Latex gloves and disposable sterile collection sticks were used to avoid contamination.

Samples from all 34 identified adult individuals were obtained by following these individuals in the field until they defecated. Samples were opportunistically collected from unidentified subadults, juveniles, and infants (107 samples) and from two neighboring groups (24 samples). The latter were collected in order to try to include potential fathers of offspring in my group. 78 of these samples were duplicates, some of which were acquired in 2014 and 2015 to replace samples for individuals for whom DNA had not yielded sufficient microsatellite results the previous years.

4.2 DNA Extraction

DNA was extracted from 201 samples. This included 190 of the samples I had collected (53 of the duplicate samples were not needed) and 11 kinda baboon samples originating from Kasanka National Park, Zambia (courtesy of Anna Weyher), from which 7 yielded sufficient DNA. These latter samples were used to compare the mitochondrial haplotypes and Y-markers in the Ngoma hybrid baboons to those found in "pure" kinda individuals. While the evolutionary origin of baboons is complex and choosing individuals to serve as genetic (or phenotypic) representatives of their species is not certain, these 11 kinda samples, along with other kinda and grayfoot genotypes obtained from Dr. Andrew Burrell at NYU mentioned later in this dissertation (from regions external to this hybrid zone) are used in this study as proxies for unhybridized individuals of each species and will therefore be referred to as "pure" kinda and "pure" grayfoot throughout this dissertation.

For a majority of the samples, the following protocol was used. A 200ul sub-sample from each sample was extracted using the Qiagen Stool Extraction Kit according to the manufacturer's instructions with a few minor modifications. After adding the Buffer ASL in Step 2, the samples incubated for 5 minutes at room temperature. The samples were centrifuged at full speed (14,000RPM) for 6 minutes rather than 3 minutes in Step 6 to pellet stool particles and inhibitors bound to the InhibitEX tablet. Finally, the samples were left to incubate for 20 minutes (instead of 1 minute) and centrifuged for 2 minutes (instead of one minute) to elute the DNA in Step 18. For the 2014 and 2015 samples, the above protocol was slightly modified (in order to increase the concentration of the DNA) by using a 300ul, sub-sample, rather than a 200ul sub-sample in Step 1. In Step 18, 75ul of elution buffer (rather than 200ul) was applied to the extraction column

and the buffer was allowed to remain in the column for 20 min before the 2 minute centrifugation; then a second elution was done in the same fashion but using only 50ul of elution buffer. The concentration of DNA in extract was measured by using a nanodrop spectrometer to ensure that DNA was present in the extract.

4.3 Mitochondrial DNA Methods

Mitochondrial DNA is known to mutate more rapidly than traditional nuclear genes, but more slowly than nuclear microsatellite markers (simple tandem repeats). This often makes mitochondrial DNA the marker of choice when undertaking studies comparing organisms at the species or subspecies level (e.g. phylogeographic studies). The mitochondrial d-loop (otherwise known as the 'control region') is known to be the fastest mutating region of the mitochondrial genome and can also be useful in population genetic level studies. Given the goals of my study, it was necessary to use d-loop sequences to assess the distant ancestry of individuals in the group. These results could then used in conjunction with the Y-microsatellite marker genotypes in the same manner as in Jolly et al [2010] to assess Y/mitochondrial discordance in the group (i.e. instances of hybridization evidenced by males possessing Y-markers of one species but mitochondrial haplotypes of the other species). In addition, due to the maternal inheritance of mitochondrial DNA, these d-loop results could reveal migration patterns in this hybrid group. In most savanna baboon species, including yellow and chacma baboons, females are philopatric and males migrate. However, since no published results exist regarding the migration patterns of kinda baboons, it is unclear whether to expect this same pattern in this kinda-grayfoot hybrid group.

4.3.1 Long Range PCR Amplification and Purification

The DNA from 104 samples (including all adult samples as well as the most reliable infant, juvenile, and subadult samples) was amplified by polymerase chain reaction (PCR) using long range PCR amplification methods and specifically designed primers. A 'long range' PCR protocol was used to amplify a 4326 base pair (bp) region of the mitochondrial genome, even though only a 429 bp sequence of the d-loop was desired. This long range strategy was followed in order to minimize the amplification of mitochondrial pseudogenes in the nuclear genome ('numt') which are known to exist in primates [Triant & DeWoody, 2007]. Since it is difficult to get long amplicons from fecal DNA (which is highly fragmented and degraded compared to DNA from blood or tissue), the size of the amplicon chosen was 4326 bp. This size segment was easier to amplify than a longer segment, but somewhat increased the chances of it being a nuclear pseudogene. I designed the primers used for PCR amplification using Primer 3 [Rozen & Skaletsky, 2000] (Table 11).

Table 11: PCR and Cycle Sequencing Primers

Long Range PCR Primers	
LR14416F	CGGCGCCTCCATACTATTTA
LR2242R	ATTCGGAGGTTTGTTTGTGC
Cycle Sequencing Primers	
L15437	CTGGCGTTCTAACTTAAACT
H15849	GTAGTATTACCCGAGCGG
Quantitative PCR Primers	
CMYC_E3_F1U1	GCCAGAGGAGGAACGAGCT
CMYC_E3_R1U1	GGGCCTTTCATTGTTTTCCA

Microsatellite Primers

D14s306F	AAAGCTACATCCAAATTAGGTAGG
D14s306R	TGACAAAGAAACTAAAATGTCCC
D11s2002F	CATGGCCCTTCTTTTCATAG
D11s2002R	AATGAGGTCTTACTTTGTTGCC
DYs576F	TTGGGCTGAGGAGTTCAATC
DYs576R	GGCAGTCTCATTTCCTGGAG
D5s1457F	TAGGTTCTGGGCATGTCTGT
D5s1457R	TGCTTGGCACACTTCAGG
D10s611F	CATACAGGAAACTGTGTAGTGC
D10s611R	CTGTATTTATGTGTGTGGATGG
D19s714F	ATGCCCTCTTCTGTCTCTCC
D19s714R	GCAGAGAATCTGGACATGCT
D8s1106F	TTGTTTACCCCTGCATCACT
D8s1106R	TTCTCAGAATTGCTCATAGTGC
D4s243F	TCAGTCTCTCTTTCTCCTTGCA
D4s243R	TAGGAGCCTGTGGTCCTGTT
D6s501F	CTGGAAACTGATAAGGGCT
D6s501R	GCCACCCTGGCTAAGTTACT
D6s291F	CTCAGAGGATGCCATGTCTAAAATA
D6s291R	GGGGATGACGAATTATTCACTAACT
DXs1683F	GAGTTGTGAGAAAGAGCAGTA
DXs1683R	AATGCCAGGTAACAACTTTAAG
D6s1280F	CTGAATTTAGTCAGGGGTTCC
D6s1280R	TCCATCACATGAGCAATTTC
D3s1768F	GGTTGCTGCCAAAGATTAGA
D3s1768R	CACTGTGATTTGCTGTTGGA
D2s119F	CTTGGGGAACAGAGGTCATT
D2s119R	GAGAATCCCTCAATTTCTTTGGA

Note: F or L = Forward Primers; R or H = Reverse Primers

These fragments were amplified using the Platinum Taq DNA Polymerase High Fidelity protocol. The 25ul protocol consisted of 5ul of template, 2.5ul of 10x Buffer, 0.5ul of DNTPs,

0.20ul of Platinum High Fidelity Taq, 1ul of each primer (forward and reverse), 1.0ul of MgSO4, and 13.80ul of water per reaction. The PCR was performed on a Techne thermocycler and an Eppendorf Mastercycler Ep Gradient S thermocycler. The program began with an initial 2 minute denaturation at 94˚C, followed by 35 cycles of denaturation (95˚C for 30 sec), annealing (55˚C for 30 sec), and elongation (68˚C for 4min 30sec), and a final 5 minute extension at 68˚C. The PCR protocol and parameters can also be found in Table 12 and Table 13.

Table 12: Long Range PCR Protocol

Ingredient	Concentration	Volume (ul)/rxn
H_2O		13.8
Buffer [X]	10	2.5
dNTP (mM)	10	0.5
MgSO4 (mM)	50	1.0
PlatniumTaq (U/ul)	5	0.2
F Primer (uM)	10	1.0
R Primer (uM)	10	1.0
DNA Template	unknown	5.0
Total Reaction Volume		**25.0**

Table 13: Long Range PCR Thermocycling Parameters

94˚C for 2:00
(95˚C for 0:30, 55˚C anneal for 0:30, 68˚C for 4:30) x 35 cycles
68˚C for 5:00
4˚C for infinity

4.3.2 PCR Clean Up and Cycle Sequencing

PCR products were run through a 1% agarose gel containing ethidium bromide and visualized on a UV transilluminator. Amplicons were cleaned prior to cycle sequencing using a mix of the Exonuclease I enzyme (Exo) and Shrimp Alkaline Phosphatase (SAP) such that 0.15ul of Exo (20u/ul) was added to 0.90ul of SAP (1U/ul) and 1.95ul SAP Buffer. Then 3ul of this mix was added to 7ul of PCR product. The procedure was performed on a Techne thermocycler for a digestion for 20 minutes at 37˚C followed by 15 minutes at 80˚C to inactivate the enzymes.

Two primers previously used in Burrell [2009] were used to cycle sequence the mitochondrial fragment of interest (420bp): forward (L15437): CTGGCGTTCTAACTTAAACT and reverse (H15849): GTAGTATTACCCGAGCGG (Table 11). Cycle sequencing reactions were performed using BigDye Terminator version 3.1(Applied Biosystems) in the following manner. The master mix included 1.50ul of 10x buffer, 1.00ul of Big Dye 3.1, 1.25ul of 10X primer, and 5.25ul of Millipore water for each 10ul reaction and then 9ul of this mix was combined with 1ul-3ul of each PCR product. Cycle sequencing was performed on either a Techne thermocycler or an Eppendorf Mastercycler Ep GradientS thermocycler, using the following protocol. The program began with a denaturation of 96˚C for one minute, followed by 35 cycles of denaturation (96˚C for 10 sec), annealing (50˚C for 5 sec), and elongation (60˚C for 4 min). The cycle sequencing protocol and parameters can also be found in Table 14 and Table 15.

Table 14: Cycle Sequencing Protocol

Ingredient	Concentration	Volume (ul)/rxn
H_2O		4.25
Buffer [X]	5	1.50
Big Dye 3.1[X]	2.5	1.00
Primer (uM)	10	1.25
DNA Template	unknown	2.00
Total Reaction Volume		**10.00**

Table 15: Cycle Sequencing Thermocycling Parameters

96˚C for 1:00
(96˚C for 0:10, 50˚C anneal for 0:05, 60˚C for 4:00) x 35 cycles
4˚C for infinity

Cycle sequencing products were then purified via Ethanol Precipitation and suspended in HiDi formamide (Applied Biosystems) and sequencing was carried out on an ABI 3130 DNA Analysis System. The Ethanol Precipitation protocol can be found in Appendix 1 (page 226). Since fecal DNA can sometime produce degraded signals, most templates were sequenced in both directions. Occasionally, clean sequences could only be produced in one direction. When this happened, they too were included. In most cases, a consensus sequence was produced for the entire region of interest; however, in a few cases, only part of the fragment could be sequenced, so a shorter consensus sequence was used.

4.3.3 Sequence Editing and Alignment

Nucleotide bases were identified automatically using Sequence Analysis 3.4 (Applied Biosystems) but were then imported into Geneious 7.1.5 (Biomatters Ltd.) for contig assembly. I checked each contig by eye for base calling errors, trimmed each to a reference sequence, and created a consensus sequence. Consensus sequences were then aligned using either the MUSCLE Alignment algorithm or the Geneious Alignment algorithm in Geneious 7.1.5 [www.geneious.com, Kearse et al., 2012].

4.3.4 Taxon Specific Mitochondrial Haplotypes

Phylogenetic trees were created in Geneious 7.1.5 (Biomatters Ltd.) to help illustrate and confirm the kinda versus grayfoot haplotypes in my group. While most phylogenetic analyses use parsimony, maximum likelihood, and Bayesian analyses for increased confidence in tree topology when undertaking phylogenetic studies, I simply wanted to assess and visualize the number of unique haplotypes present, as well as identify which individuals in this group possessed kinda versus grayfoot haplotypes. I therefore used both UPGMA and neighbor joining trees for my analysis.

4.4 Quantitative PCR Methods

DNA quantification was carried out in two ways. First, the amount of total DNA in each genomic sample was quantified using a nanodrop to ensure that a measurable amount of DNA

was present in each extraction. However, since genomic extracts of fecal samples can have many types of DNA in them (i.e. mitochondrial, nuclear, plant, bacterial, etc.) and since nanodrop technology overestimates this DNA concentration, I performed quantitative PCR (qPCR) in order to quantify the amount of nuclear baboon DNA in each sample. I carried out a 5' nuclease assay that targets an 81-bp portion of the c-myc proto-oncogene from mouse (Accession no. X01023) and human (Accession no. M38057) using methods described in Morin [2001] and modifications used in the NYU Anthropological Genetics Lab (Burrell, pers. comm.). The primers used were: forward primer (CMYC_E3_F1U1) GCCAGAGGAGGAACGAGCT and reverse primer (CMYC_E3_R1U1) GGGCCTTTCATTGTTTTCCA (Table 11). This assay was performed using the protocol for 15ul reactions from the NYU Anthropological Genetics Lab with 1.5ul of template, 7.5ul ABI Power SYBR Green PCR Master Mix, 1.5ul 10X BSA, 0.3ul of each primer (forward and reverse), and 3.9ul of Millipore water (Table 16). PCR amplification was performed using a BioRad CFX Connect Real Time qPCR thermocycler using the following protocol: initial incubation of 10 min at 95ºC, followed by 50 cycles of 95ºC for 15 sec and 59ºC for 60 sec (Table 17). A 10ng/ul TaqMan Control Genomic Human DNA standard was obtained from Life Technologies (by request; order number 4312660) and a portion of it was diluted to result in a 1ng/ul standard. Duplicate sets of these standards of known DNA amount (10ng/ul and 1ng/ul) were included with each set of samples. The c-myc 5' nuclease assay was used to quantify the amount of amplifiable single-copy DNA present in 83 fecal samples. These amounts were used to determine how many microsatellite replications were necessary to obtain reliable genotypes in light of genotyping errors such as allelic dropout [Arandjelovic et al., 2009].

Table 16: Quantitative PCR Protocol

Ingredient	Concentration	Volume (ul)/rxn
H_2O		3.9
SYBR Green	2	7.5
BSA	10	1.5
F Primer (uM)	10	0.3
R Primer (uM)	10	0.3
DNA Template	unknown	1.5
Total Reaction Volume		**15.0**

Table 17: Quantitative PCR Thermocycling Parameters

95˚C for 10:00
(95˚C for 0:15, 59˚C anneal for 1:00)x 50 cycles
4˚C for infinity

Following recommendations by Arandjelovic et al. [2009], two replicates should be carried out for samples with >101pg of DNA/rxn, three replicates for 51-100pg/rxn, four replicates for 26-50pg/rxn, and five replicates for <25pg/rxn. Based upon these recommendations, Table 18 describes how many replicates were needed. I ended up carrying out at least these recommended number of replicates.

Table 18: Number of microsatellite replicates needed based upon categories in Arandjelovic et al. [2009].

Amount of DNA/rxn (in pg)	No. of replicates needed	No. of samples fitting each category
>101	2	8
51-100	3	3
26-50	4	7
<25	5	65

4.5 Microsatellite Genotyping Methods

Microsatellites, also known as simple tandem repeats (STRs) or simple sequence repeats (SSRs) are sequences in the nuclear genome two to six base pairs in length that repeat themselves multiple times. When microsatellites evolve, they usually do so by adding or subtracting one or more of these two to six base pair repeat motifs [Estoup et al., 2002]. This phenomenon is due to unequal crossing over during recombination [Richard & Pâques, 2000; Burrell, 2009] or DNA slippage during replication [Eisen, 1999]. Microsatellite markers are known to mutate rapidly (at a rate of 10^{-2} to 10^{-6} events per locus per generation) and are thought to evolve neutrally, but evidence suggests that some may be functional and therefore be under selective pressure [Li et al., 2002]. Because microsatellites have this fast mutation rate, are most likely selectively neutral, and are abundant in the genome, they are very useful nuclear markers for population genetic studies.

All microsatellite loci used in this study are known to amplify and vary in baboons and are known to amplify using primers that are human MapPairs [Woolley-Barker, 1999; Buchan et al., 2003, 2005; Burrell, 2009; Moscovice et al., 2009; Charpentier et al., 2012; Snyder-Mackler

et al., 2012]. Since this study examines the relatedness of baboons within the same hybridizing group with only a few samples from a nearby group and from individuals of each "pure" species from outside the region, a relatively large number of loci was needed to get an adequate sense of the nuclear genetic variation in the population. Some loci were more variable than others, making them optimal for both ancestry and parentage-related analysis. Loci that are less variable are likely more suitable for assessing the ancestry in hybrid individuals and loci that are more variable may be more informative for assessing parentage. Twelve of the 14 loci are autosomal and one each is on the X and Y chromosome. The complete list of loci, primers, and multiplex sets can be found in Table 19. A forward and reverse primer was used for amplification of each locus. Both unlabeled and labeled versions of the forward primer and unlabeled versions of the reverse primers were ordered in order to carry out the approach discussed below.

Table 19: Microsatellite Loci, Primers, and Primer Sets

Locus	Dye	Set ID	Repeat Size	T_m	Estimated Fragment Size**
D14s306	NED	SetA2	4	(57)/57	159-194/*173-189*
D11s2002	HEX	SetA2	4	51(57)/57	252-280/*252-260*
DYs576	FAM	SetA2	4	55(60)/60	245-307
D5s1457	NED	Set A3	4	58/60	110-160/*111-139*
D10s611	HEX	Set A3	4	53(55)/55	156-225
D19s714	FAM	Set A3	4	56(58)/58	237-257
D8s1106	NED	Set B	4	58/57	131-210/*132-160*
D4s243	VIC	Set B	4	59/60	146-190/*155-167*
D6s501	PET	Set B	4	54/58	171-227/*171-187*
D6s291	FAM	Set B	2	55/61	193-203/*201-219*
DXs1683	NED	Set C	2	(52)/55	145-179
D6s1280	VIC	Set C	4	58/57	163-191
D3s1768	PET	Set C	4	58/58	178-227/*183-213*
D2s119	FAM	Set C	2	59/61	212-222

*paraentheses indicate T_m of dyed forward primer; rest are those of unlabeled primers (Forward/Reverse)
**italics indicate expected ranges from Snyder-Mackler based upon gelada baboons;
other ranges listed are estimates from Burrell 2009 on Zambian baboons

4.5.1 2-Step Multiplex Approach

In order to genotype my samples, I used a 2-step multiplex PCR approach [Arandjelovic et al., 2009; Orkin, 2014] which has proven to be successful when genotyping primates using feces-derived DNA. When used in combination with quantitative PCR, it can reduce the number of replicates needed per sample (up to 7) in order to reduce genotyping error [Taberlet et al., 1996] and thereby also help conserve both genomic DNA and funds.

This method involves two distinct rounds of multiplex PCR. Multiplex PCR reactions involve the amplification of multiple loci at once, compared to standard PCR (which only amplifies a single locus). For both rounds of multiplex PCR, the Qiagen Multiplex PCR kit was used and the PCR thermocycling was performed on either a Techne thermocycler or an Eppendorf Mastercycler Ep GradientS thermocycler. For both rounds of multiplex PCR, I used a touchdown protocol adopted from Dr. Snyder-Mackler [pers comm.] with the following parameters: initial 15-minute denaturation at 95˚C, followed by12 cycles of denaturation (95˚C for 30 sec), annealing (65˚C for 1min 30 sec) (-1 degree/cycle), and elongation (72˚C for 1min). This was then followed by another 35 cycles of denaturation (95˚C for 30 sec), annealing (53˚C for 1 min 30 sec), and elongation (72˚C for 1min), and a final 30-minute extension at 60˚C (Table 20). The basis behind this touchdown protocol is that by starting with a high annealing temperature (65˚C), only the specific regions of interest will amplify, thereby reducing unintended non-specific DNA that might amplify otherwise under lower temperatures [Korbie & Mattick, 2008]. This annealing temperature is reduced by one degree for each of 12 cycles, decreasing the specificity each cycle, but at the same time continuing to primarily amplify what amplified in the previous "more specific" rounds. By cycle 12 and for the remaining 30 cycles, the annealing temperature is low (53º C) to increase the ease at which these already amplified regions create copies in the remaining cycles.

Table 20: UMPCR and MPCR Thermocycling Parameters

Touchdown Protocol
95˚C for 15:00
(95˚C for 0:30, 65˚C anneal for 1:30 (-1 degree/cycle), 72˚C for 1:00) x 12 cycles
(95˚C for 0:30, 53˚C anneal for 1:30, 72˚C for 1:00) x 30
60˚C for 30:00
4˚C for infinity

4.5.2 Unlabeled Multiplex PCR (UMPCR)

The initial multiplex reaction, referred to as UMPC (Unlabeled Multiplex PCR) was carried out using the unlabeled versions of all primers for the 14 loci in a single reaction (24 unlabeled primers in total). I created 50ul of Unlabeled Primer Mix by combining 1.0ul of each of the 24 stock primers with 26ul of Millipore water [Orkin, 2014]. I then used the Qiagen Multiplex PCR kit to carry out the multiplex PCR reactions. Each 10ul reaction consisted of 5.0ul of Qiagen Mulitplex Mix, 1.0ul of Q Solution, and 0.5ul of primer mix, and 3.5-4.5ul of DNA template (Table 21).

Table 21: UMPCR Protocol (first round PCRs)

Ingredient	Concentration	Volume (ul)/rxn
Multiplex Mix	10	5.0
Primer Mix*	10	0.5
Q Solution	50	1.0
DNA Template	unknown	3.5-4.5
Total Reaction Volume		**10.0**

4.5.3 Primer Sets and Labeled Primer Mixes for Labeled Multiplex PCR

I divided the 14 loci into four sets. Two sets consisted of 3 loci each, with the fluorescently dyed forward primers belonging to the 4-dye set (NED, HEX, FAM, ROX size standard) as in Burrell [2009] and two sets each consisted of 4 loci with the fluorescently dyed forward primers belonging to the 5-dye set (NED, VIC, PET, FAM, LIZ size standard). Loci were assigned to a particular set such that each locus within a set differed significantly in size, and forward primers for each locus were given a different colored fluorescent tag so alleles for each locus could be read separately when genotyped in the ABI 3130 DNA Analysis System. These sets were based upon sets previously used by Burrell [2009] and Snyder-Mackler [per comm.] that were already known to amplify together in harmony under a particular set of conditions. Primer mixes (50ul) were created for each of these four primer sets as described above. 1.0ul of each unlabeled reverse primer in the set was combined with 1.0ul of each labeled forward primer in the set, and enough Millipore water to reach a total volume of 50ul (e.g. for a 4 primer set, one would add 1.0ul of each of 4 unlabeled reverse primers, 1.0ul of each of 4 labeled forward primers, and 42ul of Millipore water for a total volume of 50ul) [Orkin, 2014].

4.5.4 Labeled Multiplex PCR (MPCR)

Each UMPCR from above then underwent a 1:100 dilution and was then used as the DNA template for the next round of multiplex PCRs. This round of multiplex PCRs, referred to as MPCR (labeled Multiplex PCR)—the more "standard" multiplex approach—generally involves a set of 3-5 loci being amplified at once (Table 19). As mentioned above, each locus

possesses two primers, a labeled forward primer (with a fluorescent tag) and an unlabeled reverse primer. I followed the Qiagen Muliplex PCR kit instructions but I used a 10ul reaction (instead of 50ul) and varied the amount of template used as necessary. Each 10ul reaction consisted of 5.0ul of Qiagen Mulitplex Mix, 1.0ul of Q Solution, 0.5ul of the primer mix (described in section 1.5c), 0.5ul of Millipore water, and 3.0-6.0ul of DNA template (Table 22). The PCR thermocycler conditions were the same as those used for the UMPCRs (Table 20).

Table 22: MPCR Protocol (2nd round PCR of particular Primer Sets)

Ingredient	Concentration	Volume (ul)/rxn
H_2O		0.5
Multiplex Mix	10	5.0
Primer Mix*	10	0.5
Q Solution	50	1.0
DNA Template (UMPCR 1:100 dilution)	Unknown	3.0-6.0
Total Reaction Volume		**10.0**

4.5.5 Genotyping

After amplification, samples were prepared for genotyping on the ABI 3130 DNA Analysis System. A mixture of 8.75ul of HiDi Formamide (Applied Biosystems) was combined with 0.25ul of Gene Scan 500 size standard (Applied Biosystems). Gene Scan 500 ROX was used as the size standard for Sets A2 and A3 and Gene Scan 500 LIZ was used for Sets B and C. This mixture was vortexed and pipetted into wells in a 96-well plate, then sealed, centrifuged, and placed in an Eppendorf Mastercycler Ep GradientS thermocycler at 95ºC for 5 min and then

flash frozen in an ice bucket until loading on to the ABI 3130 DNA Analysis System. Each individual was genotyped between 3 and 7 times at each locus following Taberlet et al. [1996].

4.5.6 Scoring Alleles

Obtaining accurate genotypes from non-invasively collected samples can be difficult due to several types of genotyping error, a few of which involve alleles failing to amplify and others which involve improperly identifying alleles. Allelic dropout and null alleles are two sources of genotyping error that are a result of alleles failing to amplify, causing heterozygotes to be scored as homozygotes. Allelic dropout is the tendency for only one of two heterozygote alleles to amplify during PCR [Taberlet et al., 1996] As this genotyping error is random, either allele has an equal chance of failing to amplify. However, this error arises more often when using samples with low DNA concentration, so to combat this, each locus must be amplified many times [Taberlet et al., 1996; Arandjelovic et al., 2009; Burrell, 2009]. The problem of null alleles also results in only one of two heterozygote alleles amplifying during PCR. However, in this case, it is due to mutations in PCR priming sites preventing certain alleles from amplifying [Pemberton et al., 1995; Dakin & Avise, 2004]. Null alleles are rarer and this issue can be detected by testing to see if the locus in question is out of linkage and Hardy-Weinberg equilibrium [Burrell, 2009].

In addition to the above sources of genotyping error, electrophoretic artifacts can lead to alleles being improperly identified due to contamination and stutter. Fecal DNA extracts contain not only DNA of the species being studied, but also DNA from plant, parasite, and bacterial

DNA. These types of DNA "contamination" may be amplified in addition to or instead of the intended DNA target, resulting in electrophoretic peaks that appear to be legitimate alleles from the species under study. Electrophoretic 'stutter' peaks arise when the Taq polymerase slips during PCR, resulting in slight size variations in the PCR product [Litt et al., 1993; Burrell, 2009]. Luckily, each locus has a distinct electrophoretic profile. When the locus is amplified and genotyped multiple times and the elecrophoretic profile is carefully examined and compared across replicates by eye, genotypes can usually be correctly scored. All alleles were scored by eye using GeneMarker v. 4.0 (SoftGenetics, LLC).

4.5.7 Genotyping Challenges

Fecal samples often contain low quality and highly fragmented DNA which makes genotyping difficult and expensive (due to the number of times each sample must be replicated (up to 7 times) [Taberlet et al., 1996]. Samples from the identified adult males, females and infants were prioritized relative to those from opportunistically collected juveniles or those that were from unknown individuals. Therefore, only samples that had worked in the sequencing process, those that represented each of the identified adult individuals and infants, and a few juvenile samples that amplified well in initial microsatellite amplifications were prioritized for genotyping at the 14 microsatellite loci. Microsatellite amplification was attempted for 101 samples, when including samples from identified adults that had to be recollected, extracted, and genotyped again, as well as samples that were thought to be from unique individuals but ended up being duplicates. A much smaller number of samples had enough complete genotypes to be used in the ancestry and parentage analyses.

4.5.8 Y-Marker Methods

These samples were also genotyped for the Y-marker (DYs576), but alleles were only expected for the adult males. Females were also genotyped at this locus as a negative control to verify that the genotypes being scored indeed belonged to the Y-marker. All infants and juveniles were also scored at this locus but any individual that came up negative multiple times was assumed to be female. I determined the Y-marker ancestral category (kinda or grayfoot) for these 21 males using the method in Jolly et. al. [2010] where all individuals with an allele greater than 280 base pairs in length were assigned kinda and those with alleles less than 280 base pairs were assigned grayfoot.

4.5.9 Autosomal Loci Challenges

Nine of the 12 autosomal microsatellite loci were chosen for use in the parentage analysis in CERVUS described later in this chapter. Three loci (Set C) that did not amplify well in enough individuals or were dinucleotide markers and were too difficult score reliably (DXs1683, D3s1768 and D2s119), were dropped from both the parentage and STRUCTURE analyses (Table 23). Forty-six individuals had complete enough genotypes at these 9 loci to be included in both the exclusion analysis and parentage analysis in CERVUS, as they had been reliably scored at more than the required 5 loci.

In the STRUCTURE analysis described later in this chapter, only 7 of the 12 autosomal loci were used. Five of these loci were the same as in the parentage analysis (Table 23). Three

loci (D8s1106, D4s243, D6s501) that would otherwise have been included in this ancestry analysis (and were included in the parentage analysis), were not included because the "pure" kinda and "pure" grayfoot samples obtained from Burrell [unpublished], necessary for the analysis, had not been genotyped at these loci. The X-marker and Y-marker were excluded because these sex-linked markers are haploid and the STRUCTURE program only allows diploid markers to be incorporated into the analysis. In addition, many of the kinda and grayfoot genotypes that I acquired from Burrell [2009] did not include these loci. Sixty-nine individuals, including 45 from my hybrid group (a few which differ from those in the parentage analysis), 11 from "pure" kindas and 13 from "pure" grayfoots were included in the STRUCTURE analysis. All other samples contained too many missing data at these 7 loci. The resulting dataset included 32 individuals with complete genotypes at all 7 loci, as well as 30 individuals missing genotypes at 1 locus, 4 missing at 2 loci, and 3 missing more than 2 loci.

In the STRUCTURE analysis, a few of the 11 "pure" kinda genotypes obtained from Burrell contained genotypes at locus D11s2002 and D19s714 that were difficult to interpret because they are tetranucleotide loci (repeat of 4) and while the two alleles of any one individual were 4 bases apart at these loci, the alleles for some individuals were only two bases apart from other individuals at these loci (i.e. some individuals were scored 260 and 264 and others 262 and 266). In order to conservatively deal with this (as I did not want these to be assessed as novel alleles), I created two datasets. In the first dataset, I shifted the genotypes of these individuals at these two loci forward by two (+2); in the second, I shifted them backwards by two (-2). I carried out the analysis using both datasets and after realizing that the direction of the shift did

not affect the GHIS for my individuals, I arbitrarily chose the +2 case for use in downstream analyses.

Table 23: Microsatellite loci used in Parentage and STRUCTURE analyses

Locus	Dye	Set ID	Used in Parentage Analysis	Used in STRUCTURE analysis
D14s306	NED	SetA2	Yes	Yes
D11s2002	HEX	SetA2	Yes	Yes
DYs576	FAM	SetA2	No; Y-marker	No; Y-marker
D5s1457	NED	Set A3	Yes	Yes
D10s611	HEX	Set A3	Yes	Yes
D19s714	FAM	Set A3	Yes	Yes
D8s1106	NED	Set B	Yes	No*
D4s243	VIC	Set B	Yes	No*
D6s501	PET	Set B	Yes	No*
D6s291	FAM	Set B	Yes	Yes
DXs1683	NED	Set C	No; X-marker**	No; X-marker**
D6s1280	VIC	Set C	No	Yes
D3s1768	PET	Set C	No**	No**
D2s119	FAM	Set C	No**	No**

*These loci were not amplified in the "pure" species samples obtained from Burrell; thus these loci could not be included in the analysis.
**These loci did not amplify well and thus were not used in the analyses.

4.5.10 Parentage Analyses

In order to determine parentage of infants (and juveniles), I carried out both an exclusion and CERVUS likelihood analysis. Exclusion is a simple approach to parentage analysis (when there are few candidate parents and highly polymorphic loci) whereby the genotypes of

candidate parents are compared against the offspring's genotype (taking account of the other parent's genotype, if available) [Kalinowski et al., 2007]. If a mismatch occurs at one or more loci individuals are excluded as parents. When there are a large number of candidate parents and/or few loci or loci that are less polymorphic, it is common that multiple candidate parents remain non-excluded and there is no way to know which non-excluded candidate parent is the true parent. In such cases, likelihood methods using CERVUS can be used to statistically distinguish non-excluded candidate parents (thereby identifying the true parent), as it takes into consideration the frequency of the offspring alleles that could have come from a candidate parent, and whether or not the candidate parent is heterozygous or homozygous [Kalinowski et al., 2007].

Of the 46 individuals included in the parentage analysis for both the exclusion and CERVUS analysis (Table 24), 15 were males (out of 16) and 16 were females (out of 20). These 15 males included 12 (of 13) identified adult males in the group, 1 adult male that transferred from the group during my fieldwork (Solomon), and 2 unidentified males that were suspected sub adults. One adult male, Kuyipa, was unable to be genotyped and thus could not be included in the analysis. The 16 females included 12 (of 14) known mothers, 2 (of 3) potential mothers (observed to be pregnant but infants were never seen) and 1 sample from an unknown female. One other complete genotype was not used in the analysis because the animal was nulliparous at the time of the study and therefore not a candidate mother. While all efforts were made to genotype all identified adult females, I was unable to include 2 remaining known mothers (Queenie and Merry) and 2 females that were not observed to be mothers at the time of the study (Chilonda, and Jesmine). Their genotypes, as well as Helena's, which were included in the

genotype file and listed as a potential mother) were too incomplete to be used in the analysis, having a large number of partial genotypes which resulted in too many missing data (Chapter 5), even after I collected new fecal samples for these individuals, re-extracted both new and old samples, created more concentrated DNA extracts, carried out a variety PCR protocols, and used different amounts and dilutions of PCR products at various stages in the process. The remaining samples consisted of 15 offspring, including 3 juveniles and 12 infants.

4.5.11 Paternity Exclusion Analysis

Genotypes were obtained for 15 of 16 potential fathers and 15 offspring (Chapter 5). Paternity was assessed for the 15 offspring in two ways: (1) paternity exclusion and (2) maximum likelihood-based paternity assignment in CERVUS 3.0.7 [Kalinowski et al., 2007]. In the paternity exclusion analysis, paternity was assigned to a male if he could not be excluded at 8 of the 9 loci and if all other males could be excluded at multiple loci.

4.5.12 Maternity Exclusion Analysis

Genotypes were obtained for 14 of 17 potential mothers and 15 offspring. Maternity was assessed in the same two ways as the paternity analyses: (1) exclusion and (2) maximum likelihood-based maternity assignment in CERVUS 3.0.7 [Kalinowski et al., 2007]. In the exclusion analysis, maternity was assigned in the same manner as in the paternity analysis.

Table 24: Table of candidate fathers and mothers and whether they were used in the CERVUS analysis

Candidate Father	Used in Analysis	Candidate Mother	Used in Analysis
Big K	Yes	April	Yes
Chifupi	Yes	Arwen	Yes
Jack	Yes	Daisy	Yes
Kink	Yes	Flora	Yes
Mavuto	Yes	Helena	Yes
Old Kuyipa	Yes	Jean	Yes
R. Simmons	Yes	Minnie	Yes
Solomon	Yes	Ndona	Yes
Spock	Yes	Selala	Yes
Strider	Yes	Yang	Yes
Uno	Yes	Unknown	Yes
Valentino*	Yes	Yin	Yes
Yogi	Yes	Zelda	Yes
Subadult male01*	Yes	Ophilia*	Yes
Subadult male02*	Yes	Rosy*	Yes
Kuyipa of 2013	No	Queenie	No
		Merry	No
		Chilonda*	No
		Jesmine*	No
		Gaga*	No

** Individuals not suspected of being a mother or father due to being too young or due to observational data detailing that the infant sample was collected before the female gave birth*

4.5.13 Parentage Analysis using CERVUS 3.0.7

CERVUS uses maximum likelihood methods to assign paternity and/or maternity based upon the genotypes of the offspring and all possible parents, and a simulation that assigns

confidence to these results. The maximum likelihood-based parentage analysis was carried out using CERVUS 3.0.7 with corrected likelihood equations [Kalinowski et al., 2007] and the error rate was set to three different levels (0.01, 0.05, and 0.10) to evaluate the robustness of the analysis. This implies that the genotyping accuracy was 99%, 95%, and 90% respectively. For all three analyses, the CERVUS simulations were run assuming 16 candidate fathers (including the 12 known adults and 3 likely subadults that were included in the analysis and 1 male that transferred from the group that was not included (15/16 = 93.8%)) and 17 candidate (possible) mothers (including 12 known mothers, 2 possible mothers that were included in the analysis and 2 known mothers and 1 unlikely but possible mother that was not included (based upon observational data (14/17 = 82%)). It was estimated that 92% of the loci were typed, based upon the Allele Frequency results in CERVUS. The necessary confidence LOD scores for parentage assignment were obtained by simulating parentage for 100,000 offspring based upon allele frequencies derived from the population. LOD scores are obtained by taking the natural log of the overall likelihood ratio. If positive, the candidate parent is more likely to be the true parent than not; if zero, the candidate parent is equally likely to be the true parent as not the true parent; if negative, "the parent" is less likely to be the true parent than the true parent [Kalinowski et al., 2007].

4.5.14 Ancestry

In order to genetically assess the ancestry of my hybrid group relative to "pure" kinda and "pure" grayfoot individuals, a STRUCTURE analysis was carried out using 45 individuals from this hybrid group and 24 individuals from "pure" kindas and "pure "grayfoots outside the

hybrid zone (Chapter 5; page 113) (Table 31). The 11 "pure" kinda individuals were from Chunga (n=8) and Kitwe (n=3) (Figure 1) [Burrell, unpublished], and the 13 "pure" grayfoot individuals were from Moremi in Botswana [Burrell, 2009]. I genotyped two of the "pure" kinda individuals; the remaining genotypes from the "pure" individuals were obtained from Dr. Burrell (Chapter 5; page 113).

4.5.15 STRUCTURE Analysis

I used the program STRUCTURE which uses a Bayesian clustering algorithm with Monte Carlo Markov chaining (MCMC) to assess population subdivision. This program is useful for identifying distinct genetic populations, identifying admixed individuals, and assigning individuals to populations. STRUCTURE uses multilocus genotypic data (and the allele frequencies at each locus) and various *a priori* assumptions of the number of populations (K) that are assumed to be randomly mating to test each of these assumptions. It does this by generating posterior probabilities that the data can be clustered into each of the K population scenarios. Because this group was suspected to be a kinda-grayfoot hybrid group, I predicted that the number of populations (K) for my dataset containing kinda, grayfoot, and hybrid individuals would be K=2, with the pure kinda and grayfoot populations being subdivided and the hybrid individuals consisting of different proportions of each.

While the highest value of the likelihood of K (L(K)) produced from the STRUCTURE results generally indicates it is the most accurate value of K, multiple iterations of MCMC in STRUCTURE are required to allow for variation in the log likelihood value, leading to multiple

likelihood values for each case of K. An *ad hoc* statistic, delta K has been found to be a more reliable test for the true value of K. Delta K is equal to the mean of the absolute value of the second derivative in likelihood value for multiple iterations of K, divided by the standard deviation of the likelihood value (Delta K = m|L"(K)|/s[L(K)]. The highest of these delta K values will be the best estimate of K [Evanno et al., 2005].

I ran each analysis using an admixture model with correlated allele frequencies with a run length of 1,000,000 iterations and a burn-in length of 100,000 Markov chain Monte Carlo iterations. I ran the entire analysis from K=1 to K=8 ten times in STRUCTURE and averaged the proportion of each individual's genome over these 10 runs [Pritchard et al., 2000; Burrell, 2009]. Any missing genotypes were ignored by default. I used the computer program, Structure Harvester, to both plot the likelihood values for each K and to calculate delta K [Earl & VonHoldt, 2012]. The highest likelihood values (L(K)) and that highest delta K values indicate the appropriate K.

Once the appropriate value of K was determined, the results for that K value from the STRUCTURE analysis was input into the program, CLUMPP [Falush et al., 2003]. CLUMPP outputs a mean of the permuted matrices across all replicates of a particular K (the variation in the results from the 10 iterations of the appropriate K). This output file of mean values can then be directly input into the clustering visualization program, *distruct* 1.1 [Rosenberg, 2004].

4.5.16 Genotypic Hybrid Index Scores (GHIS)

A Genotypic Hybrid Index was created using the mean Q-matrix scores produced from the CLUMPP analysis where 0.00 = Kinda and 1.00 = Grayfoot (Table 33). Individuals were then clustered into Kinda-like, Intermediate, and Grayfoot-like categories based upon natural breaks in these ordered scores.

Chapter 5: Genetic Results

5.1 Mitochondrial DNA Results

A 429 bp region of the mitochondrial d-loop (including hypervariable region 1) was sequenced in 104 samples. These samples included all 34 identified adult males and females in my group, 50 opportunistically collected samples from probable subadults (n = 3), juveniles (n = 29), and infants (n = 9) in my group (some of which were likely redundant), 4 samples from the Elephant Orphanage group ~11km away, 10 samples from a nearby group that occasionally would merge with my group (Shy Group), and 7 pure kinda samples from Kasanka National Park (courtesy of Anna Weyher) for comparison. Two "pure" sequences were downloaded from GenBank for use in the analysis (Accession no. NC_020008 - *P. kindae* from Kasanka NP, Zambia [Zinner et al., 2013] and Accession no. JN116773 – *P. ursinus* from Livingstone, Zambia [Sithaldeen et al., unpublished]).

5.1.1 Taxon Specific Mitochondrial Haplotypes

The 104 d-loop sequences (including one grayfoot and one kinda GenBank sequence) resulted in 8 unique haplotypes (Table 25 and Figure 15). Four of these haplotypes are similar (with few base pair differences) and cluster with known grayfoot samples in both the UPGMA and neighbor joining analyses (with high bootstrap values) (Figure 15). 70 samples had the same haplotype (G1), including all but two adult females in my hybrid group, two Elephant Orphanage samples, one sample from the neighboring group, and a grayfoot sequence found on GenBank

(Accession no. JN116773) from Livingstone, Zambia (17.8500° S, 25.8667° E) (Figure 1). One Elephant Orphanage sample and 8 samples from my hybrid group carried a second haplotype (G2: n=9). A third haplotype (G3; n=13) was found in 9 of the neighboring group samples, one Elephant Orphanage sample, and three samples from my hybrid group, all of whom were identified adult males (022 - Spock, 005 - Uno, 015 - Strider). A fourth haplotype (G4; n=2) was found in only one individual from my group, an identified adult male (007_125 - Mavuto) (Table 25 and Figure 15).

The other four haplotypes cluster with known kinda samples in the both the UPGMA and neighbor joining analyses (with high bootstrap values) (Figure 15) (K1, n=3; K2, n=2; K3, n=1, K4; n=4). The 6 "pure" kinda samples from Kasanka (Figure 1) carried three of these haplotypes. The K4 haplotype was found in three Kasanka samples (KS233, KS205, KS188), as well as the kinda sequence on GenBank (Accession no. NC_020008). The K1 haplotype (n=3) was found in 2 identified adult males from my group in Kafue (011_124 - Big K and 019 – R.Simmons) and another unidentified individual from my group (095) (Table 25 and Figure 15).

The resulting kinda and grayfoot haplotypes were then used in conjunction with the microsatellite loci mentioned in the next section (particularly the Y-marker) to help determine the overall ancestry of the individuals in the group.

Table 25: List of samples with mtDNA d-loop sequences and taxon-specific haplotypes; G1-G4 = grayfoot haplotypes and K1-K4 = kinda haplotypes; Individuals with no name are referred to as NA; F = female, M = male, A = adult, J = juvenile, I = infant, U = unknown.

SampleID	Locality (Group)	Indiv Name	mtDNA Ancestry	mtDNA Haplotypes	Sex	Age
001	Ngoma	Daisy	Grayfoot	G1	F	A
008	Ngoma	Chifupi	Grayfoot	G1	M	A
009	Ngoma	Old Kuyipa	Grayfoot	G1	M	A
016	Ngoma	Kink	Grayfoot	G1	M	A
017	Ngoma	Yogi	Grayfoot	G1	M	A
018	Ngoma	Jack	Grayfoot	G1	M	A
020	Ngoma	NA	Grayfoot	G1	F	A
023	Ngoma	NA	Grayfoot	G1	F	A
025	Ngoma	Zelda	Grayfoot	G1	F	A
029	Ngoma	Juvenile01	Grayfoot	G1	M	J
034	Ngoma	NA	Grayfoot	G1	U	J
038	Ngoma	NA	Grayfoot	G1	U	J
042	Ngoma	NA	Grayfoot	G1	U	U
043	Ngoma	NA	Grayfoot	G1	U	J
044	Ngoma	NA	Grayfoot	G1	U	U
048	Ngoma	NA	Grayfoot	G1	U	U
054	Ngoma	NA	Grayfoot	G1	M	J
056	Ngoma	NA	Grayfoot	G1	M	J
060	Ngoma	NA	Grayfoot	G1	M	J
064	Ngoma	NA	Grayfoot	G1	F	J
065	Ngoma	NA	Grayfoot	G1	U	J
066	Ngoma	NA	Grayfoot	G1	F	J
068	Ngoma	NA	Grayfoot	G1	F	J
069	Ngoma	NA	Grayfoot	G1	U	J
079	Ngoma	NA	Grayfoot	G1	U	J
081	Ngoma	NA	Grayfoot	G1	U	U
082	Ngoma	NA	Grayfoot	G1	U	J
083	Ngoma	NA	Grayfoot	G1	F	J
084	Ngoma	NA	Grayfoot	G1	U	U
085	Ngoma	NA	Grayfoot	G1	M	J
087	Ngoma	NA	Grayfoot	G1	U	J
088	Ngoma	NA	Grayfoot	G1	U	J
093	Ngoma	NA	Grayfoot	G1	F	U
094	Ngoma	NA	Grayfoot	G1	U	J

SampleID	Locality (Group)	Indiv Name	mtDNA Ancestry	mtDNA Haplotypes	Sex	Age
096	Ngoma	NA	Grayfoot	G1	M	U
097	Ngoma	NA	Grayfoot	G1	U	J
105	Ngoma	NA	Grayfoot	G1	U	U
106	Ngoma	NA	Grayfoot	G1	U	U
107	Ngoma	NA	Grayfoot	G1	U	U
108	Ngoma	NA	Grayfoot	G1	U	U
109	Ngoma	NA	Grayfoot	G1	U	U
112	Ngoma	NA	Grayfoot	G1	M	U
113	Ngoma	NA	Grayfoot	G1	U	U
114	Ngoma	NA	Grayfoot	G1	M	A
115	Ngoma	Infant01	Grayfoot	G1	U	I
119	Ngoma	NA	Grayfoot	G1	U	I
122	Ngoma	NA	Grayfoot	G1	U	I
130	Ngoma	Jesmine	Grayfoot	G1	F	U
131	Ngoma	Chilonda	Grayfoot	G1	F	A
132	Ngoma	Merry	Grayfoot	G1	F	A
133	Ngoma	Kuyipa	Grayfoot	G1	M	A
137	Ngoma	Solomon	Grayfoot	G1	M	A
148	Ngoma	Juvenile02	Grayfoot	G1	U	J
151	Ngoma	Infant08	Grayfoot	G1	U	I
004_**206_077**	Ngoma	Selala	Grayfoot	G1	F	A
021_123	Ngoma	Yang	Grayfoot	G1	F	A
028_**200**	Ngoma	Minnie	Grayfoot	G1	F	A
101_**202**	Ngoma	Helena	Grayfoot	G1	F	A
111_**230**	Ngoma	Flora	Grayfoot	G1	F	A
116_**226**	Ngoma	Yin	Grayfoot	G1	F	A
12_135	Ngoma	Rosy	Grayfoot	G1	F	A
127_**204**	Ngoma	Gaga	Grayfoot	G1	F	U
134_**128**	Ngoma	April	Grayfoot	G1	F	A
140_145_**232**	Ngoma	Infant02	Grayfoot	G1	M	I
142_143	Ngoma	Juvenile03	Grayfoot	G1	U	J
203_089_**086**	Ngoma	Arwen	Grayfoot	G1	F	A
EO022	Ngoma (Ele Orph)	EO022	Grayfoot	G1	U	U
EO025TR	Ngoma (Ele Orph)	EO025TR	Grayfoot	G1	U	U
JN116773	Livingstone	GenBank	Grayfoot	G1	U	U
SG008	Ngoma (Shy)	SG008	Grayfoot	G1	U	U

SampleID	Locality (Group)	Indiv Name	mtDNA Ancestry	mtDNA Haplotypes	Sex	Age
002	Ngoma	NA	Grayfoot	G2	U	U
040	Ngoma	NA	Grayfoot	G2	U	U
120	Ngoma	Infant11	Grayfoot	G2	U	U
126	Ngoma	NA	Grayfoot	G2	U	U
128	Ngoma	NA	Grayfoot	G2	U	U
129	Ngoma	Infant09	Grayfoot	G2	U	U
039_**238**	Ngoma	Ndona	Grayfoot	G2	F	A
EO016	Ngoma (Ele Orph)	EO016	Grayfoot	G2	U	U
005	Ngoma	Uno	Grayfoot	G3	M	A
015	Ngoma	Strider	Grayfoot	G3	M	A
022	Ngoma	Spock	Grayfoot	G3	M	A
EO001	Ngoma (Ele Orph)	EO001	Grayfoot	G3	U	U
SG002	Ngoma (Shy)	SG002	Grayfoot	G3	U	U
SG003	Ngoma (Shy)	SG003	Grayfoot	G3	U	U
SG006	Ngoma (Shy)	SG006	Grayfoot	G3	U	U
SG009	Ngoma (Shy)	SG009	Grayfoot	G3	U	U
SG010	Ngoma (Shy)	SG010	Grayfoot	G3	U	U
SG011	Ngoma (Shy)	SG011	Grayfoot	G3	U	U
SG012	Ngoma (Shy)	SG012	Grayfoot	G3	U	U
SG013	Ngoma (Shy)	SG013	Grayfoot	G3	U	U
SG014	Ngoma (Shy)	SG014	Grayfoot	G3	U	U
007_125	Ngoma	Mavuto	Grayfoot	G4	M	A
011	Ngoma	Big K	Kinda	K1	M	A
019	Ngoma	R.Simmons	Kinda	K1	M	A
095	Ngoma	NA	Kinda	K1	M	A
KS184	Kasanka	Otis	Kinda	K2	M	A
KS225	Kasanka	Woody	Kinda	K2	M	A
KS216	Kasanka	Muma	Kinda	K3	M	A
NC_020008	Kasanka	GenBank	Kinda	K4	U	U
KS188	Kasanka	Natalie	Kinda	K4	F	J
KS205	Kasanka	Rhianna	Kinda	K4	F	I
KS233	Kasanka	Mowgly	Kinda	K4	M	J

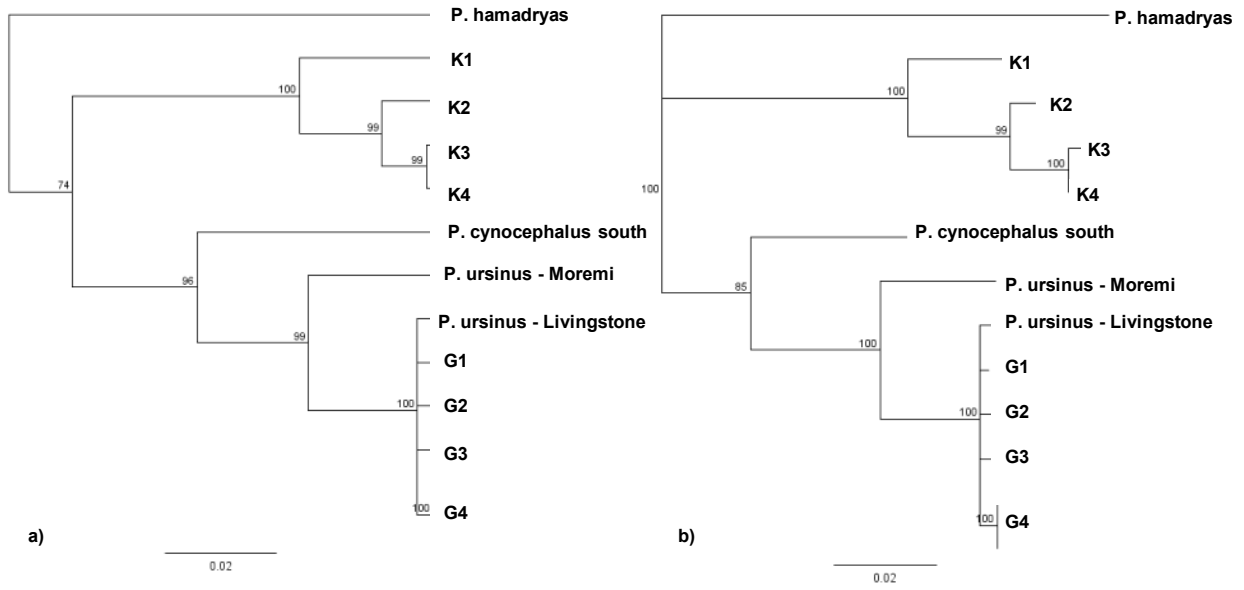


Figure 15: Phylogenetic trees of d-loop haplotypes from Table 25. a) UPGMA, b) neighbor joining. Outgroup = *P. hamadryas* (accession number Y18001.1) ; K1-K4 = kinda haplotypes; G1-G4 = grayfoot haplotypes; *P. cynocephalus* (Amani, TZ; accession number JX946200.2); *P. ursinus* (Moremi, Boswana; accession number JN116785), and *P. ursinus* (Livingstone, Zambia; accession number JN116773). Numerical bootstrap values >70 are shown above the branches.

5.2 Y-marker Results

The Y-marker (DYs576) was successfully scored in 12 (out of 13) adult males, as well as 4 subadult males and 5 male infants. I was only able to amplify the Y-marker once rather than the requisite 3 times for Jack, and I was unable to amplify the Y-marker for Chifupi (Table 26). I obtained Y-marker genotypes for 6 of 8 male "pure" kinda samples (3 from Chunga, 3 from Kasanka) (Figure 1). No Y-marker alleles were found in any of the females.

Three males (Jack, Uno, and Subadult03) possessed a kinda Y-marker, the Y-markers for the rest of the individuals were of grayfoot origin (Table 26). For infants whose paternity was successfully assessed, the Y-marker of the infant's father agreed with the Y-marker of the offspring in all cases. In Chapter 7, these Y-marker ancestral categories are used in conjunction with the mitochondrial ancestral categories to assess the extent of Y-mitochondrial discordance in this group.

Table 26: Male DYs576 alleles and Y-marker ancestral categories

Sample ID(s)	Indiv Name	Y-marker Ancestry	DYs576 Allele
011	BigK	Grayfoot	269
008	Chifupi	Unknown	Unknown
018	Jack	Kinda	291[+]
016	Kink	Grayfoot	271
133	Kuyipa	Grayfoot	265
009	Old Kuyipa	Grayfoot	278
007_125	Mavuto	Grayfoot	269
019	R.Simmons	Grayfoot	271
137	Solomon	Grayfoot	271
022	Spock	Grayfoot	271
015	Strider	Grayfoot	271
005	Uno	Kinda	297
014	Valentino	Grayfoot	265
017	Yogi	Grayfoot	265
153	Subadult01	Grayfoot	265
163	Subadult02	Grayfoot	271
155	Subadult03	Kinda	290
140_145_232	Infant02	Grayfoot	271
224_228	Infant07	Grayfoot	271
234	Infant10	Grayfoot	271
236	Infant13	Grayfoot	271

[+] *refers to sample that could only be amplified once*

[1] *Father and/or mother is based upon paternity results in later in this section.*

5.3 Parentage Analysis Results

Genotypes for 46 individuals were used in the parentage analysis (Table 27), and results for the paternity and maternity exclusion and likelihood analyses are described below.

5.3.1 Paternity Analysis via Exclusion

In the Paternity Exclusion analysis, paternity could be assigned to 14 of the 15 offspring (Table 28). Twelve of the 15 offspring could be assigned to males within the group. For two offspring, all males mismatched at two or more loci, suggesting that the fathers are either not present within the group or the father was the adult male from the group that was unable to be genotyped. Finally, one male matched at all but one typed locus; however, due to missing data he was only able to be assessed at 6 of 8 loci respectively. Thus, he is likely the father but was unable to be assigned paternity via exclusion based upon the criteria in section 4.5.11.

5.3.2 Maternity Analysis via Exclusion

Using this exclusion approach, mothers were assigned to 6 of 15 offspring (Table 29). This was because few females could be genotyped at all 9 loci. All females in which fewer than 9 loci were genotyped, were automatically unable to be matched at 8 of 9 loci using the criteria in section 4.5.12. In a few other cases, more than one female matched at all or all but one locus, thereby making it impossible to determine which of these females was the mother.

Table 27: Genotype table for paternity and maternity analyses; Column 1 = Sample ID; Column 2 = Individual name; Column 3 = the sex of the individual where F=female, M=male and U=unknown; each two columns after this indicate the 2-allele genotype for each of the 9 loci used in this analysis; 0 = missing data.

Sample	Individual	Sex	D8s1106		D4s243		D6s501		D6s291		D14s306		D11s2002		D5s1457		D10s611		D19s714	
001	Daisy	F	137	145	0	0	194	0	194	198	171	183	253	265	126	130	177	181	241	249
027	Jean	F	137	145	169	169	194	202	198	202	171	191	265	265	122	130	177	189	241	249
300	Gaga	F	137	137	161	169	186	186	198	198	175	195	261	269	112	130	173	177	249	249
302	Ndona	F	137	141	161	169	170	186	198	198	159	171	261	265	0	0	169	189	245	257
305	Helena	F	137	0	169	169	0	0	0	0	183	0	265	0	122	0	201	205	245	245
306	Ophilia	F	137	137	161	169	206	214	198	198	175	183	253	253	122	126	177	181	241	241
307	Yin	F	137	141	169	173	194	0	198	198	171	191	0	0	0	0	189	197	249	0
028_200	Minnie	F	145	145	165	173	210	210	194	198	171	0	265	0	126	130	181	0	249	0
089_203_20	Arwen	F	137	145	161	169	194	214	198	198	159	163	265	265	112	122	181	209	237	241
111_230	Flora	F	137	149	0	0	186	186	198	202	163	183	0	0	122	126	169	181	241	253
116_226_024	Unknown	F	137	141	165	169	194	214	198	198	171	191	265	273	122	122	189	197	241	249
12_135	Rosy	F	137	137	169	177	210	210	198	198	171	191	265	269	112	126	169	177	237	249
134_128	April	F	133	137	161	165	182	202	198	198	159	191	253	265	122	130	165	173	241	245
21_123	Yang	F	137	137	165	169	194	210	198	198	163	175	265	269	112	122	201	209	225	237
25_303	Zelda	F	145	145	161	169	202	210	198	202	171	191	257	265	112	122	181	201	241	257
77_004_206	Selala	F	137	137	169	169	202	206	198	202	159	183	261	265	112	138	177	201	241	257
005	Uno	M	133	133	169	173	210	214	198	200	171	183	261	261	0	0	181	189	241	253
007	Mavuto	M	133	137	169	181	202	218	198	198	171	0	269	269	122	130	209	221	241	245
008	Chifupi	M	145	149	173	177	210	214	198	198	171	191	253	257	122	126	181	201	245	253
009	Old Kuyipa	M	145	149	161	161	182	202	194	198	171	191	256	261	112	122	201	201	245	257
011	Big K	M	133	137	173	0	182	210	197	201	171	175	265	269	122	130	173	201	249	0
015	Strider	M	133	137	161	169	202	210	198	200	171	179	265	269	130	130	0	0	241	245
016	Kink	M	137	137	161	165	186	214	198	198	171	175	261	277	124	124	177	193	241	245
017	Yogi	M	133	137	161	169	214	218	198	198	163	167	261	265	130	130	169	169	225	237
018	Jack	M	137	149	169	177	182	186	198	202	163	183	253	265	122	126	169	181	241	253
019	R. Simmons	M	133	137	165	173	174	214	198	198	175	175	257	261	130	134	181	201	241	245
022	Spock	M	137	137	165	173	202	206	198	198	171	175	261	265	124	126	173	181	241	241
137	Solomon	M	137	137	165	169	182	210	198	198	167	167	269	277	126	142	169	225	241	249
114	Valentino	M	137	137	169	0	182	194	198	198	171	171	265	265	0	0	189	189	0	0
153	Subadult01	M	137	0	165	169	194	210	198	198	167	191	265	265	126	0	177	205	241	253
163	Subadult02	M	137	137	173	185	194	202	198	198	171	175	0	0	122	130	177	0	245	0
115	Infant01	U	133	145	165	169	202	214	198	202	175	191	257	257	122	130	181	201	241	241
140_145_232	Infant02	U	137	137	169	169	182	210	198	198	163	167	269	277	122	126	209	225	225	249
162	Infant03	U	133	145	161	173	202	210	194	200	179	191	265	269	130	130	173	181	245	249
225_208	Infant04	U	133	141	161	169	210	210	198	200	159	179	265	265	130	130	169	189	241	245
235	Infant05	U	133	133	161	161	182	210	198	198	159	179	253	265	122	130	169	173	241	241
209_222	Infant06	U	137	149	161	177	194	202	198	200	179	183	253	265	126	130	169	177	241	245
224_228	Infant07	U	137	141	161	169	202	214	198	200	171	179	269	277	122	130	173	189	241	249
151	Infant08	U	137	145	165	169	186	210	198	198	167	183	265	277	126	142	181	225	241	241
129	Infant09	U	137	145	165	169	186	210	198	198	167	183	265	277	126	142	181	225	241	245
234	Infant10	U	141	145	165	173	194	214	198	198	163	175	257	265	122	130	181	201	237	245
120	Infant11	U	133	0	169	169	202	214	198	202	171	171	0	0	0	0	181	189	241	257
139	Infant12	U	137	149	169	177	186	206	202	202	163	171	253	269	124	130	201	213	241	241
029	Juvenile01	U	137	145	165	169	202	206	198	202	171	175	261	265	122	124	173	201	241	241
148	Juvenile02	U	137	137	161	165	186	214	198	198	171	175	261	277	112	124	177	193	241	245
142_143	Juvenile03	U	133	137	161	165	194	202	198	202	159	163	269	269	112	122	173	201	237	249

5.3.3 Parentage Analysis using CERVUS 3.0.7

The results of the 0.01, 0.05, and 0.10 parentage analyses were concordant. Only the confidence levels changed when the error rate increased such that individuals with 95% strict confidence levels only had 80% confidence when the error rate increased. In other words, as genotyping uncertainty increased, the confidence in the paternity result decreased (the result could increasingly be attributed to the genotyping).

5.3.4 CERVUS Paternity Results

Using the error rate of 0.01, paternity was assigned to 14 of the 15 offspring, 10 of them with a 95% confidence and 4 with 80% confidence (Table 28). For 2 offspring (including the juvenile to which no father could be assigned, i.e., Juvenile03) the LOD scores were negative. Any paternities with a negative LOD score and less than 80% confidence have been considered unreliable and for further analysis have been labeled “Unknown”.

5.3.5 CERVUS Maternity Results

Maternity was assigned to 13 of the 15 offspring, 6 of them with a 95% confidence and 7 with 80% confidence (Table 29). For 2 of these individuals, the LOD scores were negative. Maternities with both a negative LOD score and less than 80% confidence have been considered unreliable, and for further analyses are considered “Unknown”.

Table 28: CERVUS Paternity Results; * = 95% confidence and + = 80% confidence

Offspring Name	Candidate Father Name	Loci typed	Pair loci compared	Pair loci mismatching	Pair LOD score	Pair confidence	Paternity Assigned by Exclusion
Infant01	R. Simmons	9	9	0	4.87E+00	*	Y
Infant02	Solomon	9	9	0	8.41E+00	*	Y
Infant03	Strider	8	8	0	6.16E+00	*	Y
Infant04	Strider	8	8	0	6.52E+00	*	Y
Infant05	Strider	8	8	0	3.14E+00	*	Y
Infant07	Strider	8	8	0	3.33E+00	*	Y
Infant08	Solomon	9	9	0	7.71E+00	*	Y
Infant09	Solomon	9	9	0	7.04E+00	*	Y
Juvenile01	Spock	9	9	0	6.16E+00	*	Y
Juvenile02	Kink	9	9	0	1.05E+01	*	Y
Infant06	Strider	8	8	0	3.06E+00	+	Y
Infant10	R. Simmons	9	9	1	1.65E+00	+	Y
Infant11	Uno	8	6	0	9.84E-01	+	N
Infant12	Jack	9	9	2	-1.06E+00	+	N
Juvenile03	Big K	7	7	3	-8.79E+00		N

Table 29: Cervus Maternity Results; * = 95% confidence and + = 80% confidence

Offspring Name	Candidate Mother Name	Loci typed	Pair loci compared	Pair loci mismatching	Pair LOD score	Pair Confidence	Maternity Assigned by Exclusion
Infant01	Zelda	9	9	0	5.34E+00	*	Y
Infant02	Yang	9	9	0	5.95E+00	*	Y
Infant03	Minnie	5	5	0	4.91E+00	*	Y
Infant05	April	9	9	0	6.84E+00	*	Y
Infant11	Zelda	9	6	0	3.82E+00	*	N
Juvenile03	Yang	9	9	0	5.22E+00	*	Y
Infant04	Ndona	8	8	1	5.81E-01	+	N
Infant06	Daisy	7	7	0	1.19E+00	+	Y
Infant07	Yin	5	5	0	1.25E+00	+	N
Infant08	Minnie	5	5	0	2.12E+00	+	N
Infant10	Arwen	9	9	1	5.77E-01	+	N
Juvenile01	Zelda	9	9	0	1.72E+00	+	N
Juvenile02	Ndona	8	8	1	-2.23E+00	+	N
Infant09	Flora	7	7	0	1.24E+00		N
Infant12	Selala	9	9	3	-8.17E+00		N

5.3.6 Paternity Consensus Among Analyses

Results from the exclusion and maximum likelihood analyses were concordant. For the paternity results, used in the remaining analyses, I used the 0.01 error rate condition, which is the default in CERVUS and the one typically used in analyses of this sort [Alberts et al., 2003] (Table 28). It is also the most conservative in terms of assigning paternity to offspring at the 80% confidence level. In these results, paternities for all but one offspring (Juvenile03) were found.

5.3.7 Maternity Consensus Among Analyses

Results from the maternity exclusion analysis were inconclusive, as many of the females did not have the requisite 8 of 9 loci sufficiently genotyped to fulfill the exclusion requirements for this analysis. The maximum likelihood analysis, on the other hand, was better at resolving the maternities, since the analysis could run provided that a female had at least 5 of the 9 loci successfully genotyped (Table 29). All maternities that were assigned by CERVUS with 95% confidence and 3 out of 5 maternities assigned with 80% confidence were consistent with recorded observational data, as all females were observed with infants when the samples were collected. For 6 of the offspring, maternities were uncertain; however for 5 out of 6 of these cases, probable maternity was based on a combination of CERVUS and exclusion analyses and observational data (Table 30). The rationale for each is detailed in the footnotes of Table 30. In two of these cases, the CERVUS results (although 80% confident) were not consistent with observational data. For example, the CERVUS results suggested that Daisy or Minnie was the mother to Infant08; however, this sample was collected when neither Daisy nor Minnie had an

infant in the group. CERVUS suggested that Flora was the third most likely mother. Since Flora was a known mother at the time of sample collection, she was chosen as the mother of Infant08 for further analyses.

These types of situations likely occurred for one of three reasons. First, it is both possible and likely that there were too many missing data in the genotypes of the "true" mothers of these offspring [Kalinowski et al., 2007]. Second, it is also likely that the "true" mother was never genotyped because 3 of these samples did not amplify sufficiently to obtain genotypes that could be used in the maternity analysis and a few others were only able to be matched at 5 to 8 of the 9 total loci. In these cases, CERVUS was attempting to find a match when the "true" parent was not included in the analysis. Third, it is also possible but not likely, that the remaining "offspring" genotypes in question were not actually from infants, but may belong to juveniles or subadults whose mother and father are not in the group. This could be the case for Infant12 whose paternity and maternity were both unable to be assigned with confidence. It is unlikely in the other cases.

Table 30: Consensus Maternity and Paternity Results

Offspring Name	Used in Analysis	Consensus Mother (Cervus, Exclusion, & Observation)	Consensus Father	Collection Date	Conclusive (F/M)
Infant01	Yes	Zelda*	R. Simmons*	3/27/2013	Yes/Yes
Infant02	Yes	Yang*	Solomon*	6/20/2013	Yes/Yes
Infant03	Yes	Minnie*	Strider*	10/21/2013	Yes/Yes
Infant04	Yes	Ndona+	Strider*	7/1/2014	Yes/Yes
Infant05	Yes	April*	Strider*	7/22/2014	Yes/Yes
Juvenile01	Yes	Zelda*	Spock*	12/1/2012	Yes/Yes
Juvenile02	Yes	Ndona+	Kink*	6/25/2013	No/Yes
Infant06	Yes	Daisy+	Strider+	7/1/2014	Yes/Yes
Infant07	Yes	Yin+[1]	Strider*	7/14/2014	Yes/Yes
Juvenile03	Yes	Yang*	Unknown	6/24/2013	Yes/No
Infant08	Yes	Flora[2]	Solomon*	6/26/2013	No/Yes
Infant09	Yes	Flora[3]	Solomon*	4/8/2013	No/Yes
Infant10	Yes	Arwen+[4]	R. Simmons+	7/22/2014	No/Yes
Infant11	Yes	Unknown[5]	Uno+	3/27/2013	No/Yes
Infant12	Yes	Selala[6]	Jack+?	6/18/2013	No/No

** indicated 95% confidence in Cervus; + indicates 80% confidence in Cervus*

[1]Cervus suggested Yin+, both Yin and Jean are possibilities from the exclusion analysis and were both mothers at the time of sample collection, so either can be mothers but I have chosen to use the cervus result, Yin, as the final candidate.

[2]Cervus suggested either Daisy or Minnie+, followed by Flora, as likely candidate mothers; however Daisy and Minnie were not mothers at the time of sample collection;Queenie who was a mother at the time of collection, was unable to be included in the analysis, leaving Flora as the most likely candidate.

[3]Cervus suggested either Daisy or Minnie+, followed by Flora, as likely candidate mothers; however Daisy and Minnie were not mothers at the time of sample collection;Queenie and Helena, who were mothers at the time of collection, were unable to be included in the analysis, leaving Flora as the most likely candidate.

[4] Cervus suggested both Yang and Arwen+ as likely candidate mothers; however, since Yang has already been assigned motherhood with confidence elsewhere, Arwen is the most likely candidate.

[5] Cervus suggested Zelda was the most likely mother, but she has been assigned with more confidence elsewhere; Jean, Yin, Daisy, and Arwen are possibilities from the exclusion analysis, but the first three were not mothers at the time of the sample collection; Arwen is a likely candidate elsewhere; Helena and Queenie, who were mothers at the time of collection, were unable to be included in the analysis leaving this mother unknown.*

[6] Cervus suggested Selala and Flora+ are the most likely candidate mothers, although its support for either was weak; Helena and Queenie were also mothers at the time of sample collection but were unable to be included in the analysis. Thus, assuming that Flora is he mother for one of the other infants above, Selala is the most

5.4 Ancestry

The genotypes of 69 individuals were used in the STRUCTURE ancestry analysis (45 from my hybrid group, 11 kinda samples, and 13 grayfoot samples) (Table 31). Both the highest likelihood values, L(K), and the highest delta K values from the STRUCTURE analysis indicated that K=2 (Table 32), supporting my original prediction. The CLUMPP and *distruct* 1.1 results of the K=2 analysis can be seen in Figure 16.

Table 31: Genotype Table for STRUCTURE Analysis; Column 1 = Sample ID; Column 2 = population where 1=Hybrid group, 2=Chunga kindas, 3=Kitwe kindas and 4=Moremi grayfoots; Column 3 indicates that I am not using population information to inform these results; each two columns thereafter indicate the 2-allele genotype for each of the 7 loci used in this analysis; -9 = missing data.

SampleID	IndivName			D6s291		D6s1280		D14s306		D11s2002		D5s1457		D10s611		D19s714	
011	Big K	1	0	197	201	183	187	171	175	264	268	122	130	173	201	-9	-9
008	Chifupi	1	0	197	197	183	187	171	191	252	256	122	126	181	201	245	253
018	Jack	1	0	197	201	171	183	163	183	252	264	122	126	169	181	241	253
016	Kink	1	0	197	197	179	179	171	175	260	276	124	124	177	193	241	245
009	Old Kuyipa	1	0	193	197	183	183	171	191	256	260	112	122	201	201	245	257
007	Mavuto	1	0	197	197	171	179	-9	-9	268	268	122	130	209	221	241	245
019	R. Simmons	1	0	197	197	175	175	175	175	256	260	130	134	181	201	241	245
137	Solomon	1	0	197	197	179	183	167	167	268	276	126	142	169	225	241	249
022	Spock	1	0	197	197	183	183	171	175	260	264	124	126	173	181	241	241
015	Strider	1	0	197	199	-9	-9	171	179	264	268	130	130	169	169	241	245
153	Subadult01	1	0	197	197	-9	-9	167	191	264	264	-9	-9	177	205	241	253
005	Uno	1	0	197	199	175	179	171	183	260	260	-9	-9	181	189	241	253
114	Valentino	1	0	197	197	171	183	171	171	264	264	-9	-9	189	189	-9	-9
017	Yogi	1	0	197	197	-9	-9	163	167	260	264	130	130	169	169	225	237
115	Infant01	1	0	197	201	175	183	175	191	256	256	122	130	181	201	241	241
140 145 232	Infant02	1	0	197	197	171	179	163	167	268	276	122	126	209	225	225	249
162	Infant03	1	0	193	199	183	187	179	191	264	268	130	130	173	181	245	249
208 225	Infant04	1	0	197	199	179	183	159	179	264	264	130	130	169	189	-9	-9
235	Infant05	1	0	197	197	179	183	159	179	252	264	122	130	169	173	241	241
209 222	Infant06	1	0	197	199	183	187	179	183	252	264	126	130	169	177	241	245
224 228	Infant07	1	0	197	199	179	187	171	179	268	276	122	130	173	189	241	249
151	Infant08	1	0	197	197	-9	-9	167	183	264	276	126	142	181	225	241	241
129	Infant09	1	0	197	197	183	183	167	183	264	276	126	142	181	225	241	245
234	Infant10	1	0	197	197	171	175	163	175	256	264	122	130	181	201	237	245
139	Infant12	1	0	201	201	179	187	163	171	252	268	124	130	201	213	241	241
029	Juvenile01	1	0	197	201	183	183	171	175	260	264	122	124	173	201	241	241
142 143	Juvenile03	1	0	197	201	171	183	159	163	268	268	112	122	173	201	237	249
148	Juvenile02	1	0	197	197	-9	-9	171	175	260	276	112	124	177	193	241	245
134	April	1	0	197	197	175	183	159	191	252	264	122	130	165	173	241	245
001	Daisy	1	0	193	197	-9	-9	171	183	252	264	126	130	177	181	241	249
027	Jean	1	0	197	201	171	183	171	191	264	264	122	130	177	189	241	249
116 226 024	Yin	1	0	197	197	171	179	171	191	264	272	122	122	189	197	241	249
12 135	Rosy	1	0	197	197	171	187	171	191	264	268	112	126	169	177	237	249
021 123	Yin	1	0	197	197	171	171	163	175	264	268	112	122	201	209	225	237
077 004 206	Selala	1	0	197	201	179	183	159	183	260	264	112	138	177	201	241	257
131	Chilonda	1	0	-9	-9	167	171	162	167	-9	-9	122	142	177	205	-9	-9

SampleID	IndivName			D6s291		D6s1280		D14s306		D11s2002		D5s1457		D10s611		D19s714	
300	Gaga	1	0	197	198	-9	-9	175	195	260	268	112	130	173	177	249	249
101 202 305	Helena	1	0	197	201	-9	-9	-9	-9	-9	-9	-9	-9	201	205	245	245
028 200	Minnie	1	0	193	197	-9	-9	-9	-9	-9	-9	126	130	-9	-9	-9	-9
302	Ndona	1	0	197	197	-9	-9	159	171	260	264	-9	-9	169	189	245	257
306	Ophilia	1	0	197	197	-9	-9	175	183	252	252	122	126	177	181	241	241
089 203 020	Arwen	1	0	197	197	-9	-9	159	163	264	264	112	122	181	209	237	241
025 303	Zelda	1	0	197	201	-9	-9	171	191	256	264	112	122	181	201	241	257
EO001	Ele Orph	1	0	197	201	171	183	171	171	260	264	112	134	169	177	245	249
BZ11074	Chunga01	2	0	193	197	183	187	179	191	264	272	130	134	201	205	249	249
BZ11075F	Chunga02	2	0	193	197	183	187	-9	-9	260	264	126	134	165	205	237	249
BZ11006+2	Chunga03	2	0	193	199	179	187	171	179	268	272	130	138	-9	-9	245	253
BZ11008+2	Chunga04	2	0	199	201	175	187	187	198	264	268	130	130	-9	-9	245	253
BZ11010+2	Chunga05	2	0	199	201	175	179	179	183	272	276	134	134	-9	-9	247	251
BZ11011+2	Chunga06	2	0	195	201	179	191	183	183	268	272	122	122	-9	-9	245	253
BZ11012+2	Chunga07	2	0	199	201	179	187	186	195	264	272	126	134	-9	-9	241	257
BZ11059+2	Chunga08	2	0	199	201	175	187	175	179	272	276	134	134	-9	-9	245	253
BZ02085	Kitwe01	3	0	193	203	183	187	171	183	252	268	-9	-9	185	193	253	257
BZ02088	Kitwe02	3	0	197	203	183	183	167	183	264	272	126	134	173	185	245	253
BZ02089	Kitwe03	3	0	197	197	179	187	179	179	260	264	134	138	173	205	245	253
AG	Moremi01	4	0	197	197	175	183	163	171	264	268	152	160	189	197	237	241
AU	Moremi02	4	0	197	197	175	179	163	175	260	264	134	134	201	201	241	245
BL	Moremi03	4	0	197	197	175	179	163	175	256	256	-9	-9	197	201	237	241
CR	Moremi04	4	0	193	197	175	183	175	175	264	268	-9	-9	197	201	237	241
HL	Moremi05	4	0	197	197	179	179	171	175	260	264	-9	-9	189	197	241	245
IC	Moremi06	4	0	197	197	179	179	171	175	256	260	-9	-9	197	201	237	241
JO	Moremi07	4	0	197	197	175	179	163	171	252	260	-9	-9	189	201	237	237
LM	Moremi08	4	0	197	201	179	179	163	163	256	260	134	134	201	201	241	245
LX	Moremi09	4	0	197	197	175	179	163	171	256	264	-9	-9	189	197	241	241
RY	Moremi10	4	0	197	197	175	179	171	175	256	264	-9	-9	193	197	237	245
TH	Moremi11	4	0	197	197	179	183	171	175	264	268	-9	-9	189	201	241	241
TT	Moremi12	4	0	197	197	179	179	163	175	260	264	126	160	193	201	237	245
WA	Moremi13	4	0	197	197	179	179	163	175	256	260	-9	-9	201	201	237	241

Table 32: Comparison of K groupings; largest likelihood and Delta K scores are best

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-1602.47	0.3093	—	—	—
2	10	-1522.88	0.456557	79.59	155.26	340.066945
3	10	-1598.55	33.979773	-75.67	3	0.088288
4	10	-1677.22	33.969457	-78.67	88.15	2.594978
5	10	-1667.74	37.526619	9.48	113.21	3.016792
6	10	-1771.47	69.502391	-103.73	93.88	1.350745
7	10	-1781.32	31.563861	-9.85	68.81	2.180025
8	10	-1859.98	89.165836	-78.66	—	—

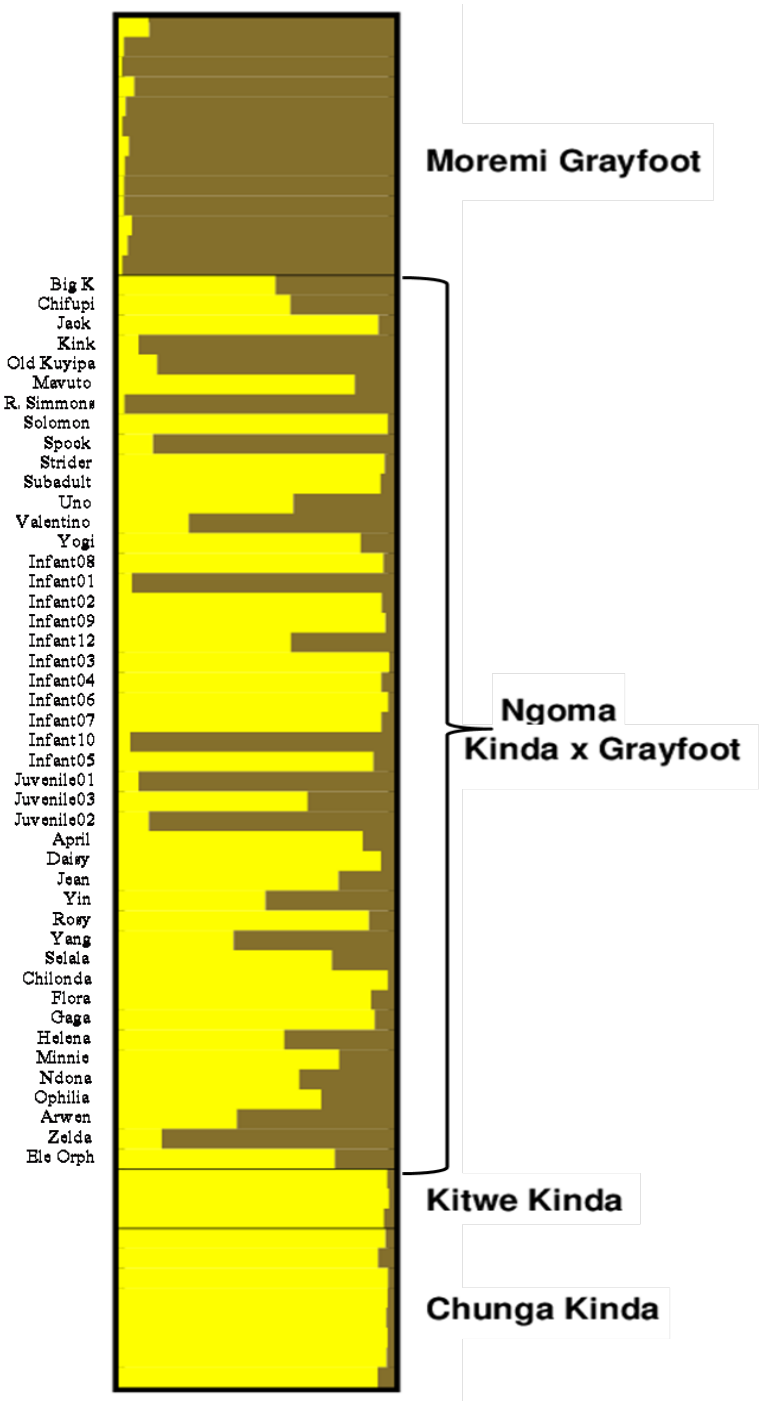


Figure 16: STRUCTURE Results indicating the proportion of kinda vs. grayfoot haplotypes each individual possesses (yellow = kinda; brown = grayfoot); Ngoma hybrids indicated by name next to their profile.

5.5 Genotypic Hybrid Index Scores (GHIS)

Table 33 lists the ordered GHI scores from the STRUCTURE analysis. Individuals from this hybrid group that fell within the range of variation for the "pure" kindas were referred to as "Kinda". Those that fell within the range of variation for the "pure" grayfoots were referred to as "Grayfoot". The remaining Kinda-like (0.00-0.25), Intermediate (0.26-0.75), and Grayfoot-like (0.76-1.00) categories have been adopted based on natural breaks in the ordered scores (Table 33). The GHIS values and categories have been graphed for all identified males (Table 34; Figure 17), females (Table 35; Figure 18), and infants (Table 36; Figure 19).

For all analyses in later chapters, which use continuous GHIS data, the GHIS values of the males, females, and infants in this group are used (Table 33). For all analyses in later chapters, which use categorical GHIS data, three GHIS categories are used: Kinda-like, Intermediate, and Grayfoot-like. All "Kinda" and "Kinda-like" individuals are grouped together into a single "Kinda-like" category and all "Grayfoot" and "Grayfoot-like" individuals are group together into a single "Grayfoot-like" category.

The GHIS values of each of the infant's parents and the GHIS categories of each of the infants's parents (which can be easily visualized in Table 37) are used in the Chapter 7 when assessing direct reproductive success by genetic ancestry. **For analyses using genotypic categories, the individuals will be coded as follows: Kinda-like = Kg, Intermediate = Ig, and Grayfoot-like = Gg**.

Table 33: Ordered Q-scores from STRUCTURE Output and GHIS Categories; "pure" kinda and "pure" grayfoots (from north Kafue and Moremi respectively) are in bold; Individuals within the range of variation of the "pure" individuals are designated with either Kinda or Grayfoot colors and each individual within the hybrid group is designated "Kinda-like", "Intermediate", or "Grayfoot-like"for further analyses.

Individual	Q-scores (K=0.00; G=1.00)	GHIS Category
Infant03	0.02	Kinda-like
Kitwe02	**0.02**	**Kinda**
Infant06	0.02	Kinda-like
Chunga03	**0.02**	**Kinda**
Chilonda	0.02	Kinda-like
Solomon	0.02	Kinda-like
Chunga04	**0.02**	**Kinda**
Chunga06	**0.02**	**Kinda**
Kitwe01	**0.02**	**Kinda**
Chunga07	**0.03**	**Kinda**
Chunga05	**0.03**	**Kinda**
Chunga01	**0.03**	**Kinda**
Infant09	0.03	Kinda-like
Strider	0.03	Kinda-like
Kitwe03	**0.04**	**Kinda**
Infant08	0.04	Kinda-like
Infant02	0.04	Kinda-like
Infant04	0.05	Kinda-like
Infant07	0.05	Kinda-like
Daisy	0.05	Kinda-like
Subadult01	0.05	Kinda-like
Jack	0.06	Kinda-like
Chunga02	**0.06**	**Kinda**
Chunga08	**0.06**	**Kinda**
Gaga	0.07	Kinda-like
Infant05	0.07	Kinda-like
Flora	0.08	Kinda-like
Rosy	0.09	Kinda-like
April	0.11	Kinda-like
Yogi	0.12	Kinda-like
Mavuto	0.14	Kinda-like
Minnie	0.20	Kinda-like
Jean	0.20	Kinda-like
Ele Orph	0.21	Kinda-like
Selala	0.23	Kinda-like

Individual	Q-scores (K=0.00; G=1.00)	GHIS Category
Ophilia	0.27	Intermediate
Juvenile03	0.31	Intermediate
Ndona	0.34	Intermediate
Uno	0.37	Intermediate
Infant12	0.37	Intermediate
Chifupi	0.38	Intermediate
Helena	0.40	Intermediate
Big K	0.43	Intermediate
Yin	0.47	Intermediate
Arwen	0.57	Intermediate
Yang	0.58	Intermediate
Valentino	0.74	Grayfoot-like
Zelda	0.84	Grayfoot-like
Old Kuyipa	0.86	Grayfoot-like
Spock	0.88	Grayfoot-like
Moremi01	**0.89**	**Grayfoot**
Juvenile02	0.89	Grayfoot-like
Kink	0.93	Grayfoot-like
Juvenile01	0.93	Grayfoot-like
Moremi04	**0.94**	**Grayfoot**
Infant01	0.95	Grayfoot-like
Moremi11	**0.95**	**Grayfoot**
Infant10	0.96	Grayfoot-like
Moremi07	**0.96**	**Grayfoot**
Moremi12	**0.97**	**Grayfoot**
Moremi05	**0.97**	**Grayfoot**
Moremi08	**0.98**	**Grayfoot**
RS	0.98	Grayfoot-like
Moremi09	**0.98**	**Grayfoot**
Moremi02	**0.98**	**Grayfoot**
Moremi10	**0.98**	**Grayfoot**
Moremi06	**0.98**	**Grayfoot**
Moremi13	**0.98**	**Grayfoot**
Moremi03	**0.99**	**Grayfoot**

Table 34: Male GHIS and GHIS Categories; GHIS are on a 0-1 scale with 0 = Kinda and 1 = Grayfoot; Kinda-like, Intermediate, and Grayfoot-like categories were created using the natural breaks in the ordered scores.

Male Name	Male GHIS	GHIS Category
Solomon	0.02	Kinda-like
Strider	0.03	Kinda-like
Subadult	0.05	Kinda-like
Jack	0.06	Kinda-like
Yogi	0.12	Kinda-like
Mavuto	0.14	Kinda-like
Uno	0.31	Intermediate
Chifupi	0.38	Intermediate
Big K	0.57	Intermediate
Valentino	0.74	Grayfoot-like
Old Kuyipa	0.86	Grayfoot-like
Spock	0.88	Grayfoot-like
Kink	0.93	Grayfoot-like
R.Simmons	0.98	Grayfoot-like

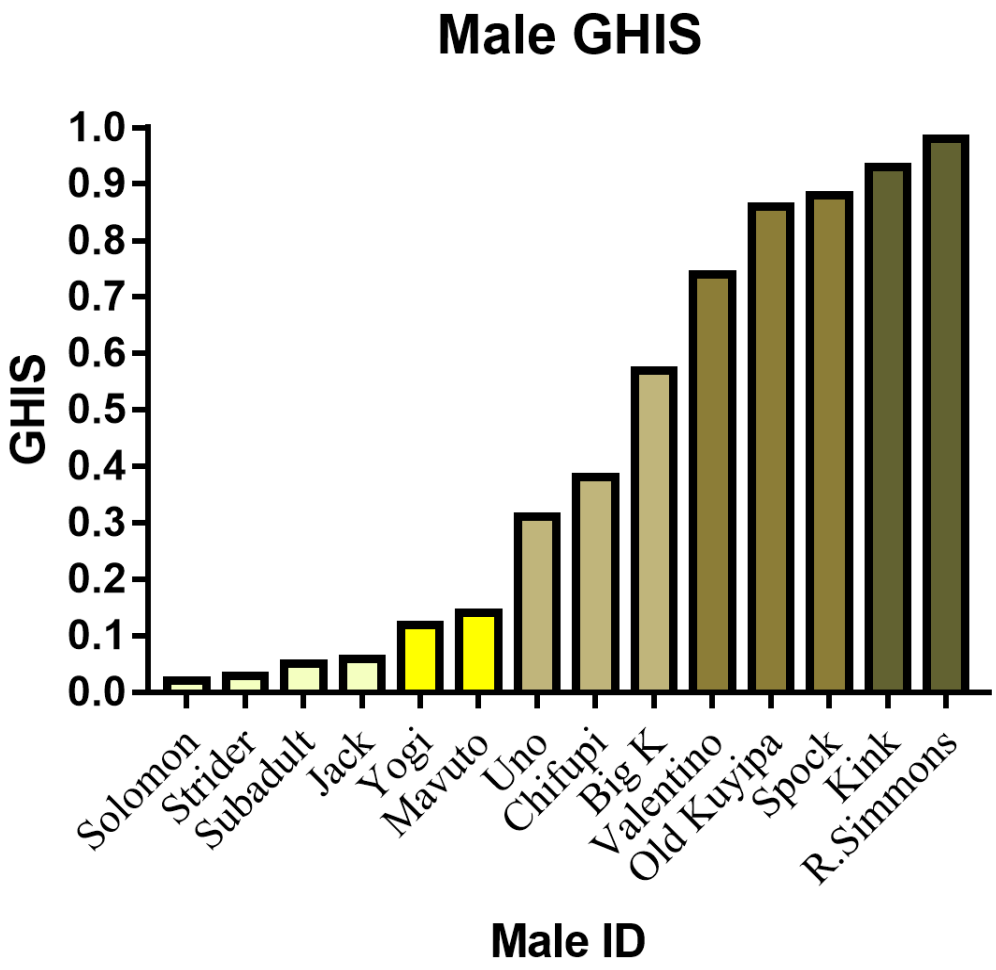


Figure 17: Male GHIS and GHIS Categories; Kinda-like (yellow; n=6), Intermediate (light brown; n=3) , and Grayfoot-like (dark brown; n=5) categories were created using the natural breaks in the ordered scores; individuals with scores that were within the range of the "pure" Kindas or "pure" grayfoots (from north Kafue or Moremi respectively) are colored as such based upon the Q-score table.

Table 35: Female GHIS and GHIS Categories; GHIS are on a 0-1 scale with 0 = Kinda and 1 = Grayfoot; Kinda-like, Intermediate, and Grayfoot-like categories were created using the natural breaks in the ordered scores.

Female Name	Female GHIS	GHIS Category
Chilonda	0.02	Kinda-like
Daisy	0.05	Kinda-like
Gaga	0.07	Kinda-like
Flora	0.08	Kinda-like
Rosy	0.09	Kinda-like
April	0.11	Kinda-like
Minnie	0.20	Kinda-like
Jean	0.20	Kinda-like
Selala	0.23	Kinda-like
Ophilia	0.27	Intermediate
Ndona	0.31	Intermediate
Helena	0.43	Intermediate
Yin	0.47	Intermediate
Arwen	0.57	Intermediate
Yang	0.58	Intermediate
Zelda	0.84	Grayfoot-like

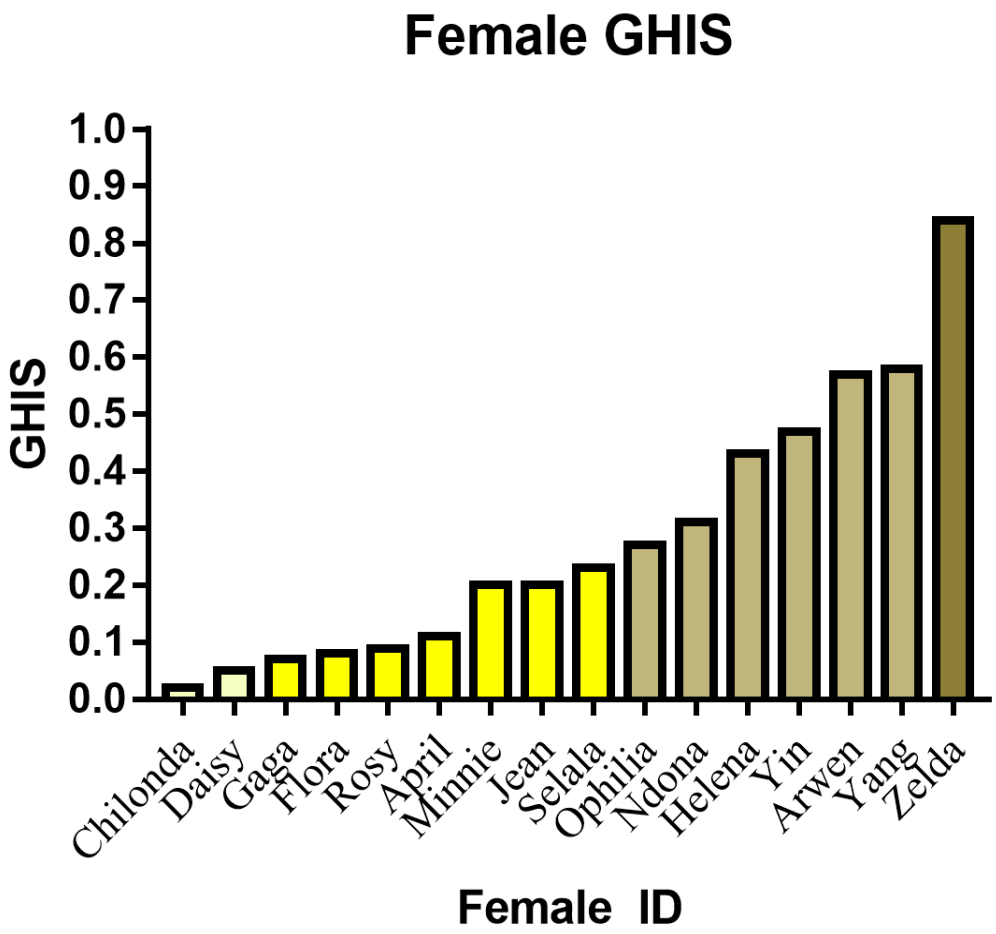


Figure 18: Female GHIS and GHIS Categories; Kinda-like (yellow; n=9), Intermediate (light brown; n=6), and Grayfoot-like (dark brown; n=1) categories were created using the natural breaks in the ordered scores; individuals with scores that were within the range of the "pure" Kindas or "pure" grayfoots (from north Kafue or Moremi respectively) are colored as such based upon the Q-score table.

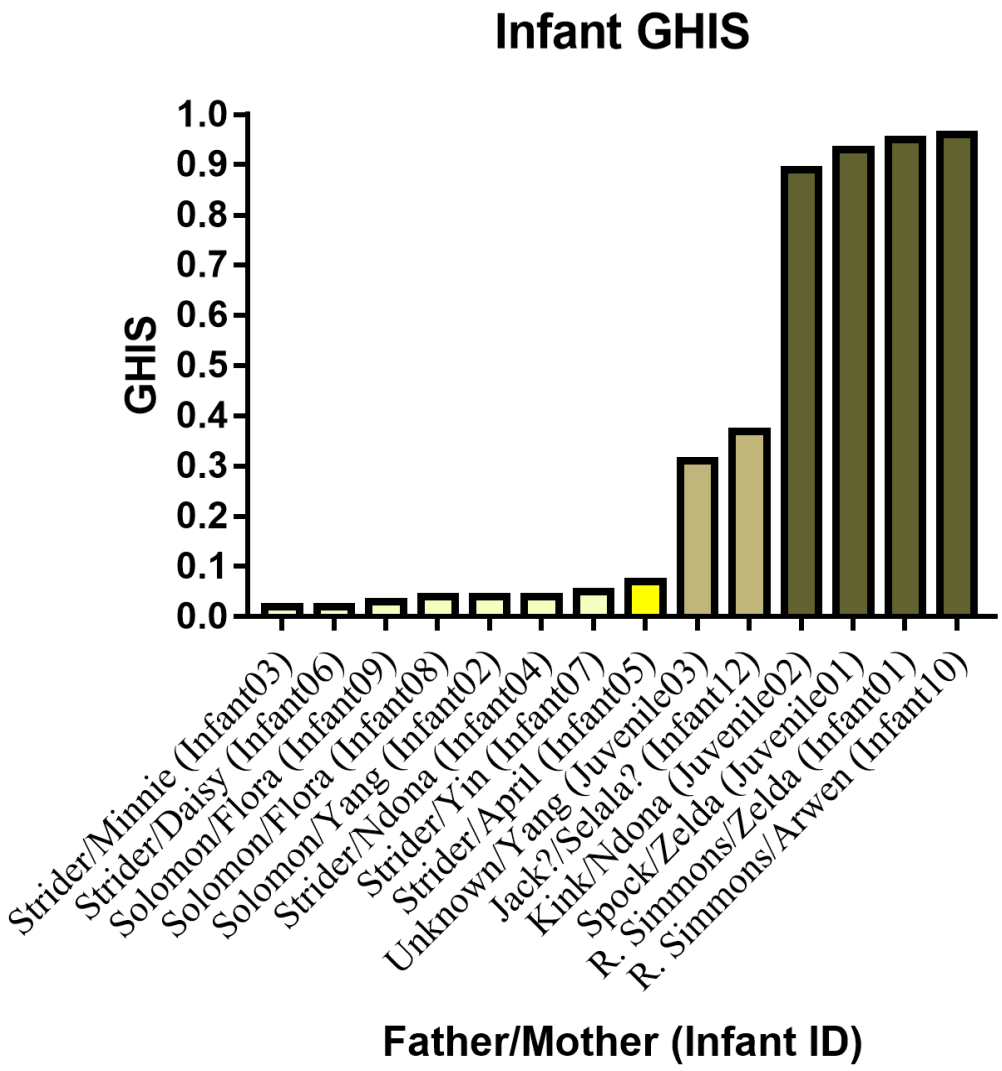


Figure 19: Infant/Juvenile GHIS and GHIS Categories; Kinda-like (yellow; n=8), Intermediate (light brown; n=2), and Grayfoot-like (dark brown; n=4) categories were created using the natural breaks in the ordered scores; individuals with scores that were within the range of the "pure" Kindas or "pure" grayfoots (from north Kafue or Moremi respectively) are colored as such based upon the Q-score table. Offspring are denoted by both their parent names and their designated infant names.

Table 36: Infant and Juvenile GHIS, GHIS Category and Parents; GHIS are on a 0-1 scale with 0 = Kinda and 1 = Grayfoot; Kinda-like, Intermediate, and Grayfoot-like categories were created using the natural breaks in the ordered scores.

Offspring Name	Father	Mother	Infant/Juvenile GHIS	GHIS Category
Infant03	Strider	Minnie	0.02	Kinda-like
Infant06	Strider	Daisy	0.02	Kinda-like
Infant09	Solomon	Flora	0.03	Kinda-like
Infant08	Solomon	Flora	0.04	Kinda-like
Infant02	Solomon	Yang	0.04	Kinda-like
Infant04	Strider	Ndona	0.04	Kinda-like
Infant07	Strider	Yin	0.05	Kinda-like
Infant05	Strider	April	0.07	Kinda-like
Juvenile03	Unknown	Yang	0.31	Intermediate
Infant12	Jack?	Selala?	0.37	Intermediate
Juvenile02	Kink	Ndona	0.89	Grayfoot-like
Juvenile01	Spock	Zelda	0.93	Grayfoot-like
Infant01	R.Simmons	Zelda	0.95	Grayfoot-like
Infant10	R.Simmons	Arwen	0.96	Grayfoot-like

Table 37: List of offspring and their parents color-coded to represent their GHIS category; yellow = Kinda-like, light brown = Intermediate, brown = Grayfoot-like.

Offspring Name	Father	Mother
Infant03	Strider	Minnie
Infant05	Strider	April
Infant06	Strider	Daisy
Infant04	Strider	Ndona
Infant07	Strider	Yin
Infant09	Solomon	Flora
Infant08	Solomon	Flora
Infant02	Solomon	Yang
Infant12	Jack?	Selala?
Juvenile03	Unknown	Yang
Juvenile02	Kink	Ndona
Infant10	R. Simmons	Arwen
Juvenile01	Spock	Zelda
Infant01	R. Simmons	Zelda

Chapter 6: Behavioral Methods

6.1 Observational Behavioral Data Collection

By February 2013, I had successfully habituated the entire focal group and individually identified all adult males and females; subadults, juveniles, and infants were not individually identifiable. I used focal sampling of all adult individuals (with a focus on estrous females) to record affiliative and agonistic social interactions (Appendix 2; page 228). To facilitate comparisons with other studies of "pure" kinda and grayfoot behavior, I adapted previously used methods for these species [Henzi & Weingrill, 1999 and Weyher pers. comm.]. Each day, I took a census of all group members (noting births, new/missing individuals, wounds), recorded infant development and female reproductive condition (as described in Chapter 3), and noted consortships. I collected these focal follow and instantaneous scan data using an Android smartphone and the *Prim8 Mobile* data collection software. Details regarding the collaboration that resulted in the creation and optimization of the *Prim8 Mobile* software are detailed in McDonald and Johnson [2014] and have been included in this dissertation as Appendix 3 (page 230). To maximize the information gathered, details of copulations and agonistic interactions by individuals other than the focal individual were also recorded when they were observed.

6.1.1 Continuous Focal Sampling and Instantaneous Scans

From February to July 2013 and again in June and July of 2014, Isaac and I conducted focal observations of all adult individuals in the group, but prioritized estrous females

(approximately 50% of the follows completed daily were on estrous females (N=<5), with the remaining follows being spread across the remaining adult male and female group members (N=>29). We used continuous 10-minute focal sampling to record specific behaviors, along with the time of occurrence and the interacting partner. These behaviors included but were not limited to reproductive behaviors, approach/withdraw behaviors, affiliative and agonistic behaviors (including grooming), adult-infant interactions, and activity budget information [Palombit et al., 1997] (Appendix 2; page 228). If a copulation involved the focal individual, the copulation was noted (with ejaculation, without ejaculation, and unknown), along with the partner, female copulation call, the individual responsible for initiating the copulation, and whether male aggression towards the female was involved [Nitsch et al., 2011]. All supplants were recorded and used to construct male dominance hierarchies as explained in the next section.

During months when the baboons were most visible (April and July) and when the baboons were still in the trees in the morning, I also conducted 2-min instantaneous scans to record the proximity among individuals (i.e. touch, <2 meters, and 2-6 meters).

From February-July 2013, Isaac and I systematically collected data on social behavior, especially behavior in the context of mating. By July 2013, we had completed approximately 4,500 10-min continuous focal follows on all 34 identified adult individuals and had collected behavioral data on 1-3 estrous cycles' worth of data for 8 different females (Figure 10 and Table 8). At that time, there were 10 females with infants of various ages and 5 pregnant females. Since then, 4 new infants were born (as noted by Issac during my 1-year absence from the field, and two females either aborted or their babies died soon after birth. Two babies with white pelage were born in 2016; these were the first "white babies" observed in this group (Table 8).

6.1.2 All Occurrence Data and *Ad Libitum* Data

In order to maximize the sample of copulations, we opportunistically recorded all observed copulations, distinguishing those that ended with ejaculation from those that did not [Nitsch et al., 2011]. In order to understand whether the unique kinda male grooming behavior affects kinda male mating success and grayfoot female mate choice, we also recorded all observed grooming bouts, recording their duration and the role of each partner in initiation and termination whenever possible [Palombit et al., 1997].

Each day, I collected *ad libitum* data including information on weather conditions, sleeping sites, general ranging patterns and environmental conditions (in addition to daily GPS track logs) (Figure 11). I also opportunistically recorded feeding preferences (i.e. information on seasonal and unique food items), and agonistic interactions that occurred too quickly to record otherwise (particularly if the observation did not include my focal individual, as these behaviors were recorded in the follow itself). Isaac and I kept a daily ongoing tally of individuals we had followed and updated each other via walkie-talkie roughly every hour to ensure that we were not watching the same individual and that we were diversifying the individuals that we followed over the course of the day.

6.2 Behavioral Data Analysis Methods

Behaviors thought to be related to reproductive success, namely grooming, male dominance rank, mate guarding, and copulations (and paternity) were the focus of the behavioral data analysis (Chapter 7 and Appendix 6). Because I had focused on estrous females, I had

recorded many more female follows than male follows. Thus, with the exception of the comparison of inter-sex grooming, in which both male and female focal follows were used, and the calculation of dominance hierarchies in which only male focal follows were used, I used only data from the 10-minute continuous focal follows of females for the majority of the analyses (Appendix 6; page 240).

Dominance rank may be assessed using displacement behaviors or using general agonistic behaviors. The Normalized David's score [de Vries et al., 2006], a matrix-based method based upon wins and losses during a particular time period, has traditionally been used to calculate a static measure of dominance rank. However, in recent years, the Elo Rating method has been adopted for use in animal behavioral research as well. It was first adopted in 1978 by Arpad Elo for use in chess and sports ratings. The major difference between these two methods is that the Elo Rating is based upon the sequence in which agonistic interactions occur and it continuously updates rankings by looking at interactions sequentially [Neumann et al., 2011]. Thus, this method can be applied as a static measure over the complete dataset or as a transient measure for any time frame within the study duration [Neumann et al., 2011]. I calculated dominance rank using only displacement behaviors and did so using both the Normalized David's Score and the Elo Rating methods (Chapter 7, page 165).

Since the behavioral data are not normally distributed, I used non-parametric methods to assess relationships between variables. Specifically, I used a Spearman's correlation for all analyses using continuous data and a Kruskal-Wallis test, Mann-Whitney U test, or Fisher Exact Test for all analyses comparing categorical data. *Post-hoc* tests were carried out where applicable and the Benjamini-Hochberg method False Discovery Rate (B-H method FDR) was carried out

when correcting for multiple comparisons. The B-H method FDR correction was used rather than a Bonferroni correction, as some have suggested that while Bonferroni corrections are good at reducing Type I error, it is too conservative, resulting in increased Type II errors. The B-H method FDR, is considered more powerful than the Bonferroni method, as it is a compromise between no correction and a Bonferroni correction that results in both reduced Type I and Type II error [Narum, 2006].

I also created a Generalized Linear Mixed Model (GLMM) to assess which factors were most influential in male mating success. The methods used in this GLMM are described below.

6.3 Generalized Linear Mixed Model (GLMM)

In order to ascertain the relative importance of each behavioral, phenotypic, or genetic factor and how together these factors influence male mating success, I created a Generalized Linear Mixed Model (GLMM). Because the behavioral data are non-normally distributed and are characterized by repeated measures, it makes it impossible to use parametric statistics for data analysis. One of the major advantages of creating Generalized Linear Mixed Models (GLMM) is that these models are more robust and powerful than simply using non-parametric statistics, yet they do not require the data to be normally distributed. They also allow the use of data characterized by repeated measures and take individual variation into account (i.e. idiosyncratic differences among males, among females, and among male-female dyads) [Winter, 2013]. Below I detail the parameters and factors used in this model, and the data used in the model can be found in Appendix 7 (page 241).

6.3.1 Poisson Distribution

The behavioral data from this study (to be used as predictor variables) can be thought of as count data. In cases of count data, there tends to be a high frequency of zeros and/or cases where there are few counts of specific behavior or event (i.e. the number of individuals or dyads who engage in a behavior or event decrease as counts of the behavior or event increase, resulting in a Poisson, rather than Gaussian, distribution). Therefore, I specified a Poisson distribution for the GLMM [Feldblum et al., 2014, Connor, pers. comm.].

6.3.2 Random Effects

Random effects are variables that are unique to Mixed Models. Models with random effects are used when one needs to account for "idiosyncratic variation due to individual differences" [Winter, 2013]. These are variables that likely affect the dependent variable in some way; however, these remain epistemic uncertainties and are not controlled for in this study.

Within this dataset, there are multiple responses from each male subject and each female subject. In such cases, these responses cannot be regarded as independent. To resolve this violation of the assumption of independence, I included both male subject (Male ID) and female subject (Female ID) as random effects in the model, allowing it to assume a different "baseline" (or intercept) for each male and each female subject [Winter, 2013] (Appendix 7; page 241).

In cases when the observed variance of the response variable is larger than expected, the data are considered to be overdispersed and the different responses cannot be regarded as

independent [Feldblum et al., 2014; Connor, pers. comm.]. To account for overdispersion in a dataset, I introduce a third random effect called "Observation", in addition to the male and female subject random effects. "Observation" accounts for the observation-specific idiosyncratic variation in my dataset by allowing the model to assume a different "baseline" (or intercept) for each "Observation" [Winter, 2013; Feldblum et al., 2014; Conner pers. comm.] (Appendix 7; page 241).

6.3.3 Fixed Effects

In linear models, Fixed Effects are the independent variables or the variables that are considered influential in the outcome of the dependent variable. In order to assess which phenotypic, genetic and/or behavioral factors were influential in mating success, I chose male dominance rank, mate guarding, grooming duration, male phenotype, and male genotype as predictor variables. Specifically, I used male dominance rank using David's Score (M_Rank_DS), mate guarding of peak estrus females (MG_Peak), male grooming duration of females of all estrus states (GrmMin_ALL), male phenotype (M_PHIS), male genotype (M_GHIS), female phenotype (F_PHIS), and female genotype (F_GHIS) as fixed effects in the model (Appendix 7; page 241). These were reproductive-related behaviors discussed in the last section that I had compared using non-parametric statistics.

Because I had an unequal number of follows for each individual and each dyad, I used the number of follows carried out on each dyad when females were in peak estrus (Foll_Peak) as

another fixed factor to help control for behavioral differences that are due to unequal observation time among individuals and among dyads.

6.3.4 Outcome Variable

Since I was interested in how factors influence reproductive success, my Outcome Variable had to be a measure of reproductive success. I used the number of copulations with peak estrous females (i.e. mating success) as an indirect measure of reproductive success, since my paternity data (a more direct measure) were temporally incongruent with my behavioral data (see Chapter 7; page 141). Mating success has been observed to be a good predictor of paternity success in some populations of baboons [Alberts et al., 2006] (Appendix 7; page 241).

6.3.5 GLMM models

To create the final GLMM model, I used different combinations of predictor variables to ascertain which would yield the most parsimonious model (within the constraint of including all variables integral to the study goals). To assess the quality of the model, I compared and contrasted AIC scores and ran a maximum likelihood ratio test/ANOVA. I removed female phenotype (F_PHIS) from the model since there did not appear to be a relationship between mating success and female phenotype in any of these models and no relationship existed between female phenotype and mating success using the non-parametric methods. I also removed female genotype (F_GHIS) from the final model for the same reason and because I did not have genotypes for all females so including this factor would have greatly reduced the overall dataset

(Appendix 7; page 241). Thus, the final model included the following factors seen in Table 38, and the results of this model are detailed in Chapter 7.

Table 38: Factors in the final Generalized Linear Mixed Model (GLMM)

Outcome Variable (Dependent Variable)	Random Effects (Idiosyncratic Independent Variables)	Fixed Effects (Predictor Independent Variables)
# of copulations of peak estrus females (Cop_Peak)	"Observation" (accounts for overdispersion) (1|Observation)	Male Dominance Rank (M_Rank_DS)
	Individual Males (1|MaleID)	Mate-guarding of peak estrus females (MG_Peak)
	Individual Females (1|FemaleID)	M:F grooming duration in minutes of females of all estrus states (GrmMin_ALL)
		Male phenotype (M_PHIS)
		Male genotype (M_GHIS)
		Number of Follows for each dyad where the female was peak estrus (Foll_Peak)

Chapter 7: Results

In this chapter, I begin by (1) exploring the relationship between phenotype and genotype in this group, (2) assessing mating asymmetry in this group, and (3) discussing the limitations of comparing direct and indirect measures of reproductive success (paternity and mating success) in this study. Using the terminology related to the individuals' phenotypic and genotypic categories specified on page 19, I integrate the genetic, phenotypic, and behavioral results from the previous chapters to test the predictions from Chapter 2 (page 19), as well as to investigate the overall factors most influential for male mating success in this group. In the following chapter (page 183), I then discuss these results in the context of the hypotheses and their predictions.

7.1 Genotype versus Phenotype

Individuals in this group possessed a range of phenotypes and genotypes (from very Kinda-like to very Grayfoot-like) that resulted in a relatively continuous distribution of GHI and PHI scores (Table 39 and Table 40). The phenotypes and genotypes of each individual are not well correlated in most cases (Table 39 and Table 40). When the relationship between PHIS and GHIS was assessed for all identified adult males and females using a Pearson's correlation, no linear correlation was found for either sex (Males:$R^2 = 0.01$; $p = 0.70$; Females: $R^2 = 0.08$; $p = 0.29$) (Figure 20 and Figure 21).

Table 39: Male GHIS and PHIS and Categories (Kinda-like, Intermediate, Grayfoot-like).

Male ID	Male Name	Male GHIS	Male PHIS	GHIS Category	PHIS Category	PHIS=GHIS? (No/Yes)
137	Solomon	0.02	0.95	Kinda-like	Grayfoot-like	No
015	Strider	0.03	0.33	Kinda-like	Intermediate	No
153	Subadult	0.05	0.18	Kinda-like	Kinda-like	Yes
018	Jack	0.06	0.84	Kinda-like	Grayfoot-like	No
017	Yogi	0.12	0.35	Kinda-like	Intermediate	No
007	Mavuto	0.14	0.93	Kinda-like	Grayfoot-like	No
005	Uno	0.31	0.00	Intermediate	Kinda-like	No
008	Chifupi	0.38	0.24	Intermediate	Kinda-like	No
011	Big K	0.57	0.15	Intermediate	Kinda-like	No
114	Valentino	0.74	0.00	Grayfoot-like	Kinda-like	No
009	Old Kuyipa	0.86	0.84	Grayfoot-like	Grayfoot-like	Yes
022	Spock	0.88	0.84	Grayfoot-like	Grayfoot-like	Yes
016	Kink	0.93	0.04	Grayfoot-like	Kinda-like	No
019	R. Simmons	0.98	0.53	Grayfoot-like	Intermediate	No

[1]*Phenotypic cutoffs: Kinda-like (<0.25), Intermediate (0.25-0.75), and Grayfoot-like (>0.75)*
[2]*Genotypic cutoffs: Kinda-like (<0.25), Intermediate (0.25-0.75) and Grayfoot-like (>0.75)*

Table 40: Female GHIS and PHIS and Categories (Kinda-like, Intermediate, Grayfoot-like).

Female ID	Female Name	Female GHIS	Female PHIS	GHIS Category	PHIS Category	PHIS= GHIS?
131	Chilonda	0.02	0.86	Kinda-like	Grayfoot-like	No
001	Daisy	0.05	0.73	Kinda-like	Grayfoot-like	No
300	Gaga	0.07	0.59	Kinda-like	Intermediate	No
111_230	Flora	0.08	0.61	Kinda-like	Intermediate	No
12_135	Rosy	0.09	0.50	Kinda-like	Kinda-like	Yes
134	April	0.11	0.59	Kinda-like	Intermediate	No
028_200	Minnie	0.20	0.86	Kinda-like	Grayfoot-like	No
027	Jean	0.20	0.59	Kinda-like	Intermediate	No
077_004_206	Selala	0.23	0.86	Kinda-like	Grayfoot-like	No
306	Ophilia	0.27	0.77	Intermediate	Grayfoot-like	No
302	Ndona	0.31	1.00	Intermediate	Grayfoot-like	No
101_202_305	Helena	0.43	0.91	Intermediate	Grayfoot-like	No
116_226_024	Yin	0.47	0.45	Intermediate	Kinda-like	No
089_203_020	Arwen	0.57	0.24	Intermediate	Kinda-like	No
021_123	Yang	0.58	0.00	Intermediate	Kinda-like	No
025_303	Zelda	0.84	0.79	Grayfoot-like	Grayfoot-like	Yes

[1]*Phenotypic cutoffs: Kinda-like (<0.50), Intermediate (0.50-0.70), and Grayfoot-like (>0.70)*
[2]*Genotypic cutoffs: Kinda-like (<0.25), Intermediate (0.25-0.75), and Grayfoot-like (>0.75)*

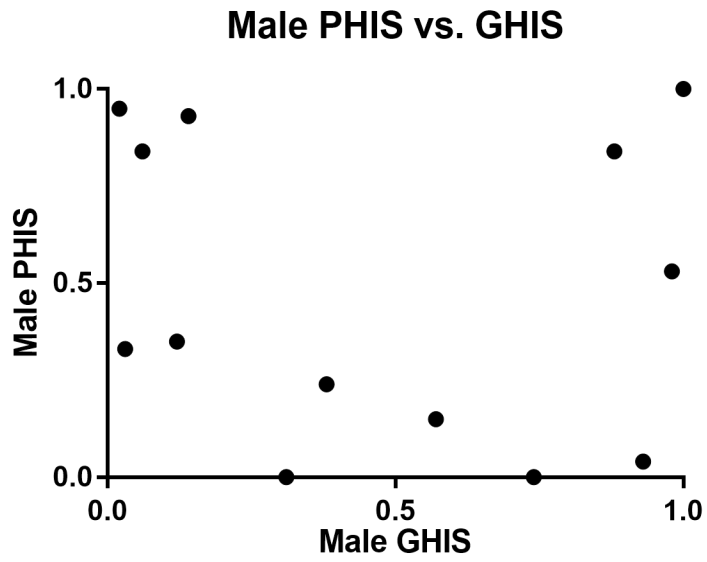


Figure 20: Male PHIS versus GHIS

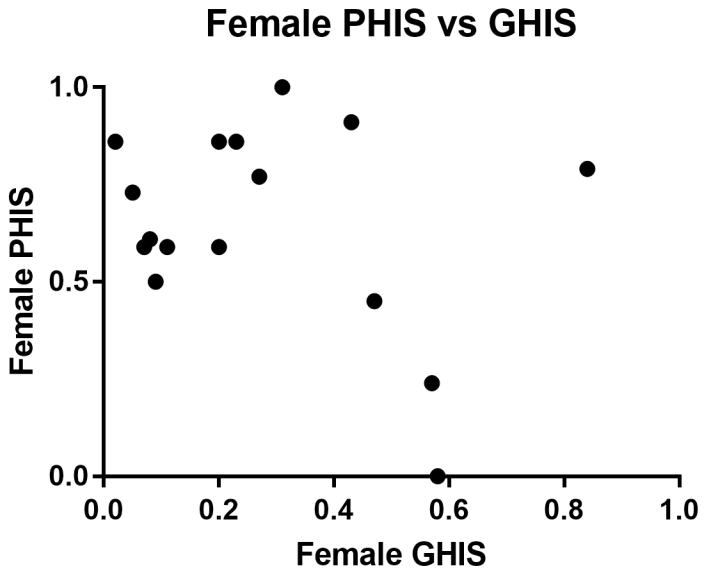


Figure 21: Female PHIS versus GHIS

Separate Pearson's correlations were carried out for each phenotypic character to assess whether any single phenotypic characteristic used in the PHIS might be related to GHIS. The only feature that was significantly positively correlated with GHIS was Nape Hair. Tail Length was found to be weakly negatively correlated with GHIS but was not significant (Table 41). In addition, I carried out a Principal Component Analysis (PCA) to see if perhaps a suite of these phenotypic characteristics might be more predictive of genetic type. However, I did not find this to be the case (Appendix 5; page 238).

Table 41: Correlations between each Male phenotypic characteristic and GHIS

Characteristics	R	95% Confidence	R^2	p-value	Sig.
Muzzle Patches	-0.065	-0.595 to 0.504	0.0042	0.833	NS
Nape Hair	0.660	0.172 to 0.888	0.4357	0.014	*
Mohawk	0.041	-0.522 to 0.579	0.0017	0.894	NS
Circumorbital Skin	-0.196	-0.674 to 0.398	0.0383	0.522	NS
Tail Carriage	-0.432	-0.794 to 0.156	0.1866	0.140	NS
Tail Length	-0.542	-0.842 to 0.012	0.2942	0.056	NS
Hair Color	0.133	-0.451 to 0.637	0.0177	0.665	NS
Body Build	0.087	-0.487 to 0.609	0.0076	0.777	NS
Body Build1	-0.137	-0.640 to 0.447	0.0189	0.654	NS
White Cheek Fur	0.075	-0.496 to 0.601	0.0057	0.807	NS
Muzzle Length	-0.149	-0.647 to 0.438	0.0221	0.628	NS

7.1.1 Discussion

GHIS and PHIS measure different aspects of an individual's genome: the GHIS is based on several unlinked loci across only a small portion of the genome; the PHIS is based upon easily observable traits whose genetic basis is unknown, but that are likely polygenic. In this study, no significant relationship was found between PHIS and GHIS.

The lack of relationship between PHIS and GHIS is likely due to the limited number of microsatellite loci used in this study and because these neutral microsatellite loci are not known to be linked to the phenotypic traits scored in this study. In the first few generations of hybridization, linkage disequilibrium is expected to produce a correlation between genetic and phenotypic traits [Bergman, 2000] that would become decreasingly correlated over time due to recombination. This pattern is observed in studies of hybrid groups in Awash National Park, Ethiopia [Bergman, 2000]. The lack of correlation between phenotype and genotype, as well as the range of phenotypes observed in the group, suggest that this hybrid zone is characterized by multiple generations of hybridization, which have allowed individuals in the group to mix sufficiently and break down linkage disequilibrium.

Previous studies [Bert & Arnold, 1995; Harrison & Bogdanowicz, 1997] have found linkage disequilibrium preserved over many generations in cases with strong selection against hybrids. In this study, as with the Ethiopian hybrids [Bergman, 2000], there is no evidence for selection against hybrids, which reproduce successfully. The dominant male in this study is the most reproductively successful, and while he is genetically Kinda-like (Kg), he is phenotypically Intermediate (Ip). In addition, none of the offspring in this study was a result of Kg x Gg or Gg x Kg pairings. Rather, they were the result of either Kg or Gg males mating with hybrid females. This suggests that baboons in this hybrid group prefer to mate with individuals genetically similar to them.

7.2 Mating Asymmetry (Y-mitochrondrial discordance)

I used fecal-derived samples to type all males in this study group using the same mitochondrial and Y-markers as those used in the original Jolly et al. [2010] study, in which the highly significant unidirectional skew towards individuals with kinda Y and grayfoot mtDNA genotypes was found. While I expected to see cases of kinda Y and grayfoot mtDNA genotypes in this hybrid group, it was also possible that cases with grayfoot Y and kinda mtDNA genotypes would also be found.

Results revealed four cases of Y-mitochondrial discordance in the 13 males in this group, but this skew was not asymmetric. Two males possessed a kinda Y-marker with grayfoot mitochondrial DNA and two males possessed grayfoot Y-markers with kinda mitochondrial DNA (Table 42). Of these males, one individual was not typed with 100% confidence, the Y-marker only amplified once.

7.2.1 Discussion

The genotyping of the 13 males in the study group reveal the first case of $Y_{grayfoot}/mt_{kinda}$ in this hybrid zone. Furthermore, these data reveal two cases of Y-mitochondrial discordance in either direction, suggesting bidirectional, rather than asymmetrical hybridization in this group. Combining the data from this study, with the data in the Jolly et al. [2010] study (n = 55), resulted in a total of 19 cases of discordance: 17 with $Y_{kinda}/mt_{grayfoot}$ genotypes and 2 with $Y_{grayfoot}/mt_{kinda}$ genotypes. Thus, while my findings showed that the hybrid zone was not

universally asymmetric across the hybrid zone, the overall pattern continues to suggest that the hybrid zone was primarily formed by kinda male and grayfoot female matings.

Table 42: Y-mitochondrial discordance

Individuals Name	Sex	Age	Ancestry (mtDNA)	Ancestry (Y-marker)	DYs576 Allele[1]	Y/mt Discord
BigK	M	A	K	G	269	**YES**
Chifupi	M	A	G	U	Unknown	UNSURE
Jack	M	A	G	K	291	**YES**[+]
Kink	M	A	G	G	271	NO
Kuyipa2013	M	A	G	G	265	NO
Old Kuyipa	M	A	G	G	278	NO
Mavuto	M	A	G**	G	269	NO
RichardSimmons	M	A	K	G	271	**YES**
Solomon	M	A	G	G	271	NO
Spock	M	A	G**	G	271	NO
Strider	M	A	G**	G	271	NO
Uno	M	A	G**	K	297	**YES**
Valentino	M	A?	G	G	265	NO
Yogi	M	A?	G	G	265	NO
Subadult01	M	J	U	G	265	UNSURE
Subadult02	M	J	U	G	271	UNSURE
Subadult03	M	J	U	K	290	UNSURE
Infant02	M	I	U	G	271	UNSURE
Infant07	M	I	U	G	271	UNSURE
Infant10	M	I	U	G	271	UNSURE
Infant13	M	I	U	G	271	UNSURE

K=kinda, G=grayfoot, U=unknown

G and G** refer to two different greyfootmtDNA haplotypes from the majority greyfoot (G) haplotype*

[+] refers to sample that could only be amplified once

[1] DSY576 alleles that were greater than 281are Kinda and those that are less than 281are grayfoot based upon Jolly et al [2010].

7.3 Paternity versus Mating Success

In this study, I measured reproductive success in two ways: paternity and mating success (number of copulations with peak estrous females). While paternity is a direct genetic measure, observed mating success has been found in some populations to be a relatively good predictor of

reproductive success in baboons [Altmann et al., 1996; Buchan et al., 2003; Alberts et al., 2006; Bergman et al., 2008], and until genetic methods were available for assessing paternity directly, it was the primary way in which reproductive success was estimated.

In this study, there was no significant relationship between mating success and the number of offspring fathered (Figure 22). One individual, Strider, had both the highest mating success and fathered the most offspring, however, there was no overall correlation between these variables.

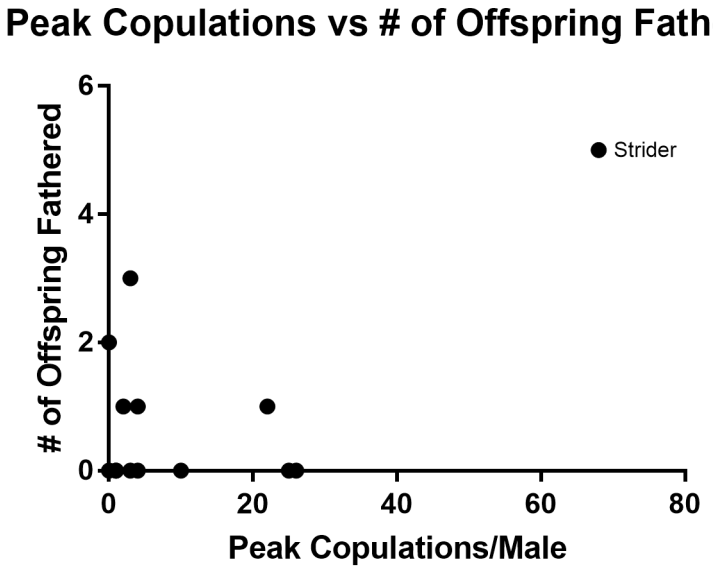


Figure 22: Relationship between measures of reproductive success. Direct measure = Number of offspring fathered; Indirect measure = number of copulations with peak estrous females. Note: behavioral data and paternity results are temporally incongruent.

7.3.1 Discussion

The results from Figure 22 may suggest that no relationship exists between mating success and paternity in this group (or that there is a single winner, while all other males are losers in this system); however, the temporal incongruence between these two variables may be leading to these results. While it would have been optimal to record the reproductive-related behaviors of the group's individuals and then, approximately a year later, collect samples from recently born infants to determine the parentage of the offspring that resulted from these behaviors, I was unable to do so due to time and funding constraints. Rather, I collected samples from the current pool of infants and at the same time collected behavioral data. As a result, the paternities are for the infants conceived prior to when the copulation behavior was recorded. Therefore, it may not be surprising that the two measures are not correlated in this study, as I observed.

While a single individual in the group had both high mating success and fathered the most infants, there was no relationship between mating success and number of offspring fathered for the other males. This makes sense in light of this temporal incongruence. For example, there were limited data on the two males in this group who fathered several offspring prior to the behavioral data collection (Solomon = 3 and R. Simmons = 2). Specifically, Solomon entered the group during the last few weeks of data collection and R. Simmons was severely injured during a fight at the beginning of the behavioral data collection. Prior to his injury, R. Simmons had appeared to be actively attempting to gain mating access to females, as predicted from his previous successful mating (n=2 offspring). After his injury though, he could barely move and/or

see, and all of his time and energy was focused on eating and resting as he recovered. Thus, the measures of mating success in these two individuals during the behavioral data collection do not shed light on the previous year's reproductive output.

Since I do not have paternities for the infants that resulted from the other reproductive-related behaviors (dominance rank, grooming and mate guarding) (as mentioned above), I have only investigated the relationship between these behaviors and mating success (as measured by the number of copulations with peak estrous females). This serves as an indirect measure of reproductive success for those portions of this study.

7.4 Test of Predictions

7.4.1 Predictions Relating to Subhypothesis A

Prediction and Summary		Support
Prediction 1	Male:Female grooming > Female:Male grooming	Partial support
Prediction 2	K-like males groom across all reproductive states	Not Supported
Prediction 3	G-like males will only groom estrus females	Supported
Prediction 4	Grooming by K-like males > G-like males	Supported
Prediction 5	Male grooming is correlated with mating success	Supported
Prediction 6	Copulation rate of K-like males > G-like males	Supported
Prediction 7	K-like males have more offspring than G-like males	Inconclusive

Group Grooming Behavior (Predictions 1, 2, and 3)

This group of baboons spent 4.3% of their total activity budget (n = 734 hours) engaged in grooming behaviors. In order to assess whether males or females groomed the opposite sex

more, I used a Mann-Whitney U test to compare the mean amount of time males spent grooming females during male follows (n = 64 follows) versus the amount of time females spent grooming males during female follows (n = 415 follows). **Prediction 1 was only partially supported.** Overall, males did not groom females more than females groomed males. Rather, there was no significant difference overall in how often the sexes groomed each other (p = 0.96) (Figure 23). Moreover, the trend was in the opposite direction than predicted. When comparing these results to those in (Figure 4), the overall grooming pattern in this group is intermediate compared to each of the "pure" species.

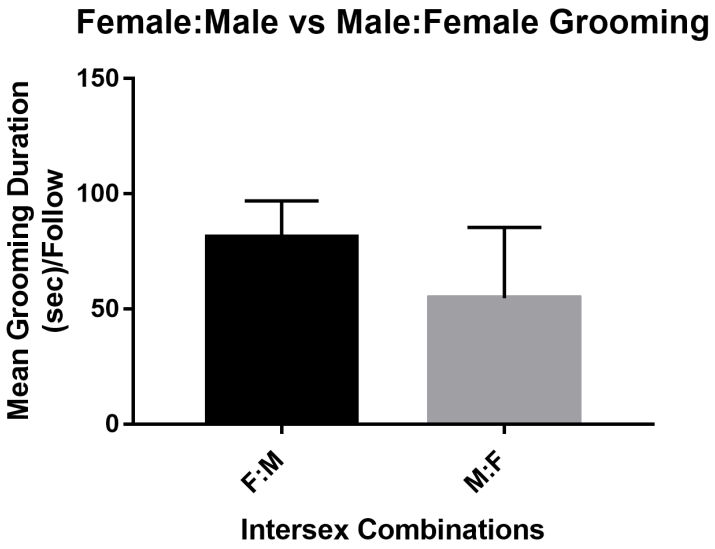


Figure 23: Inter-sex grooming: The average amount number of seconds per follow that each individual spent grooming an individual of the opposite sex.

With this said, when comparing inter-sex grooming by the phenotypic and genotypic ancestry of the groomer, results reveal that individuals are grooming in a species-specific manner. For example, Kp (and Ip) males groomed females more than Kp (and Ip) females

groomed males (Kruskal-Wallis test: p = 0.02 (p = 0.07)), while Gp females groomed males more than Gp males groomed females (Kruskal-Wallis test: p = 0.002) (Figure 24). However, due to the lack of correlation between phenotype and genotype in this group, the genotypic results differed from this. Kg (and Ig) females groomed males more than Kg (and Ig) males groomed females (p = 0.32), and since there were no Gg females, it was impossible to compare inter-sex grooming among Gg individuals (Figure 25).

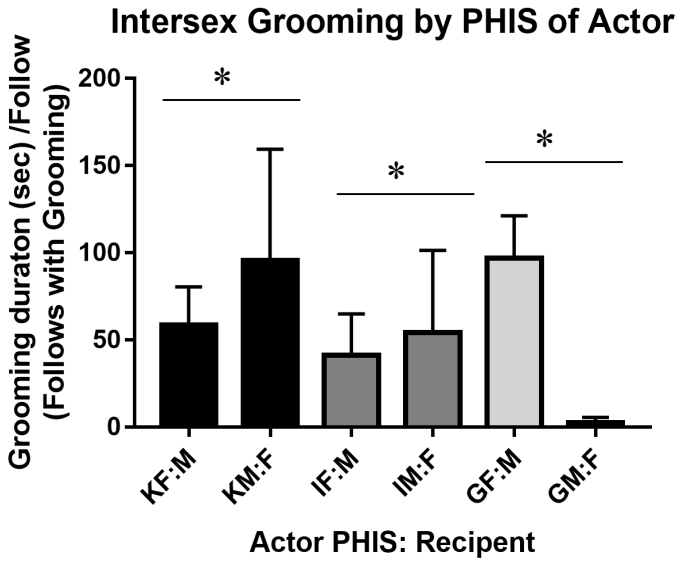


Figure 24: Comparison of inter-sex grooming by phenotype of the groomer. F = Female and M = Male; K = Kinda-like, I = Intermediate, G = Grayfoot-like.

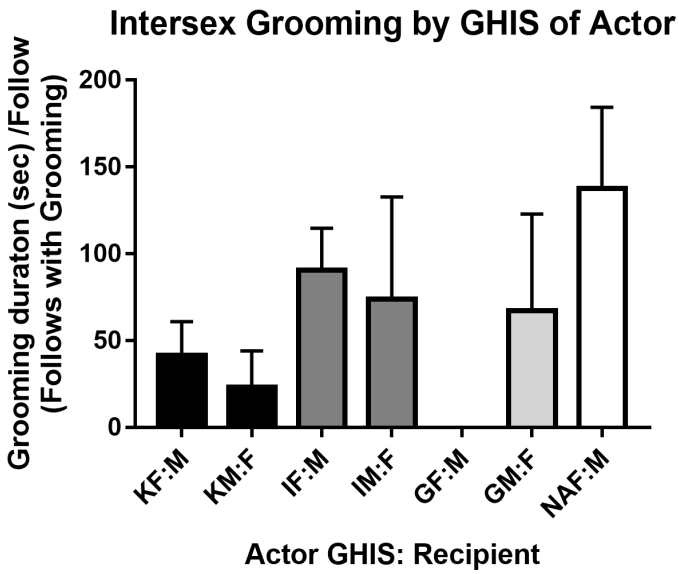


Figure 25: Comparison of inter-sex grooming by genotype of the groomer. F = Female and M = Male; K = Kinda-like, I = Intermediate, G = Grayfoot-like, NA = unknown ancestry (unable to be genotyped). The lack of GF:M grooming is result of no GF females in the group.

When looking at the group as a whole, males spent significantly more time grooming estrous females compared to non-estrous females ($p<0.0001$) (Figure 26). Breaking the group down by phenotypic and genetic ancestry did not change these results (Figure 27 and Figure 30). Kp males prioritized their estrus grooming efforts on early estrous females (Figure 28), while Gp males prioritized peak estrous females (Figure 29). Kg males increased their grooming efforts as the estrus cycle progressed (Figure 31), while Gg males groomed more early and peak, rather than mid-estrous females (Figure 32).

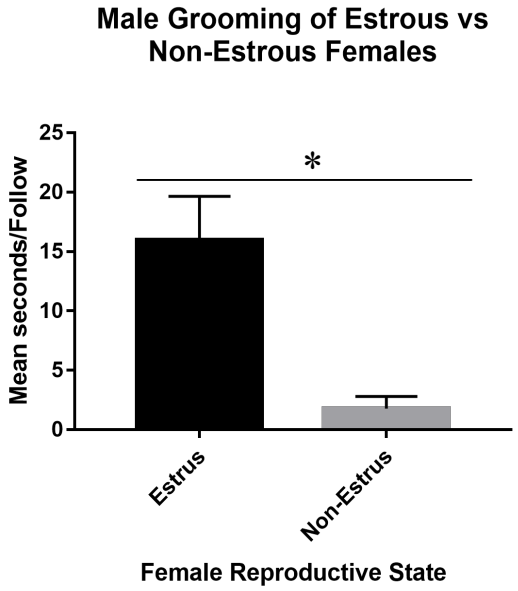


Figure 26: Male grooming duration of estrous versus non-estrous females indicates that males prefer to groom estrous females.

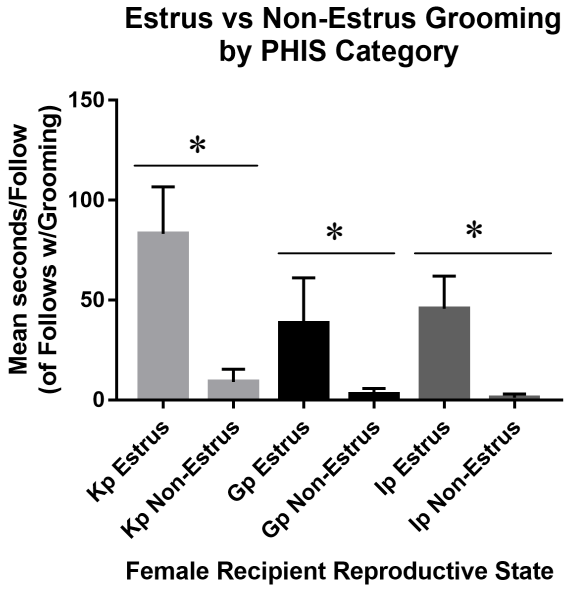


Figure 27: Estrus versus non-estrus grooming by phenotype of the male partner. Males of all phenotypic categories groomed estrous females more than non-estrous females; Kinda-like = Kp, Grayfoot-like = Gp, Intermediate = Ip.

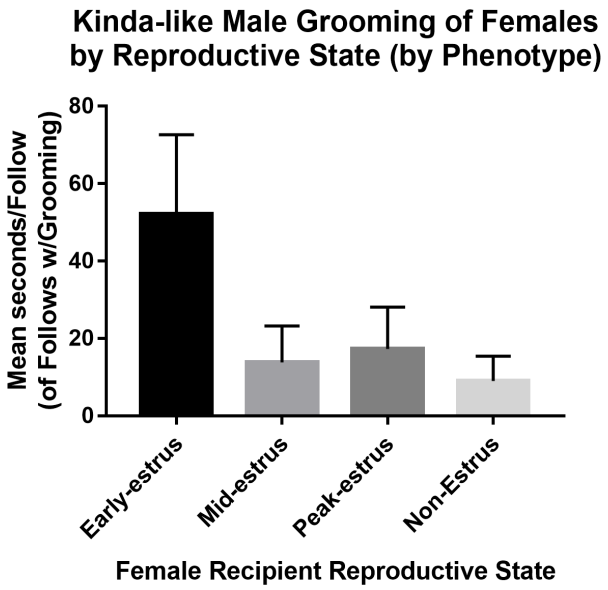


Figure 28: Kinda-like (Kp) male grooming of females by reproductive state

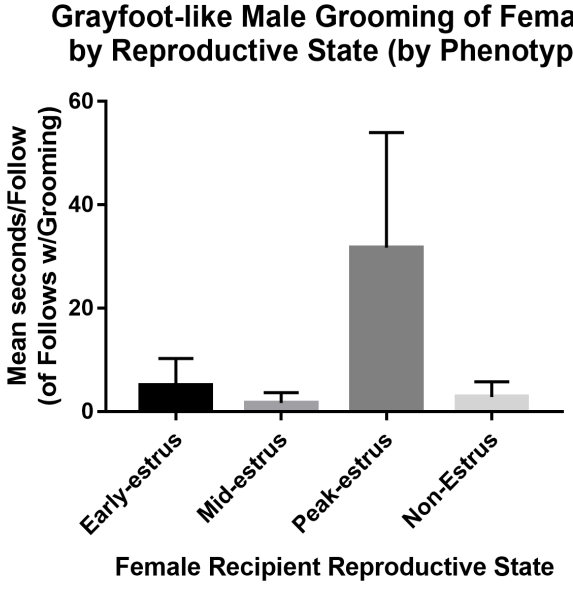


Figure 29: Grayfoot-like (Gp) male grooming of females by reproductive state

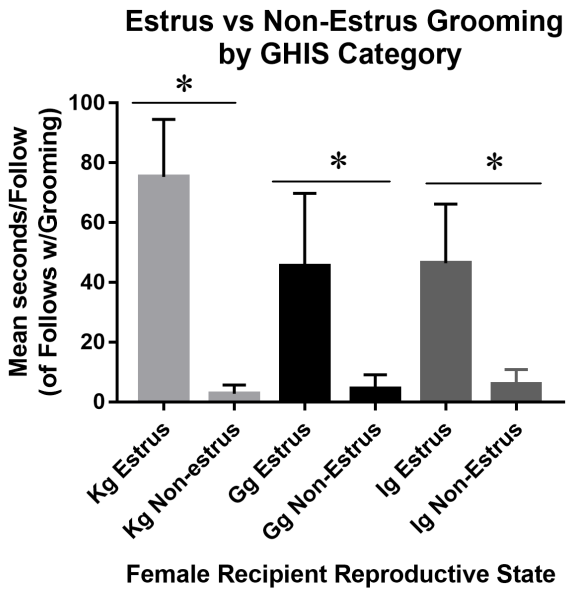


Figure 30: Estrus versus non-estrus grooming by genotype of the male partner. Males of all genetic categories groomed estrous females more than non-estrous females; Kinda-like = Kg, Grayfoot-like = Gg, Intermediate = Ig.

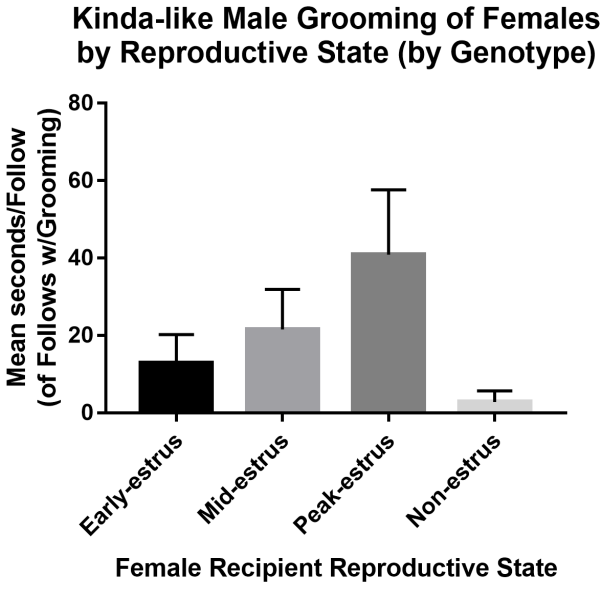


Figure 31: Kinda-like (Kg) male grooming of females by reproductive state

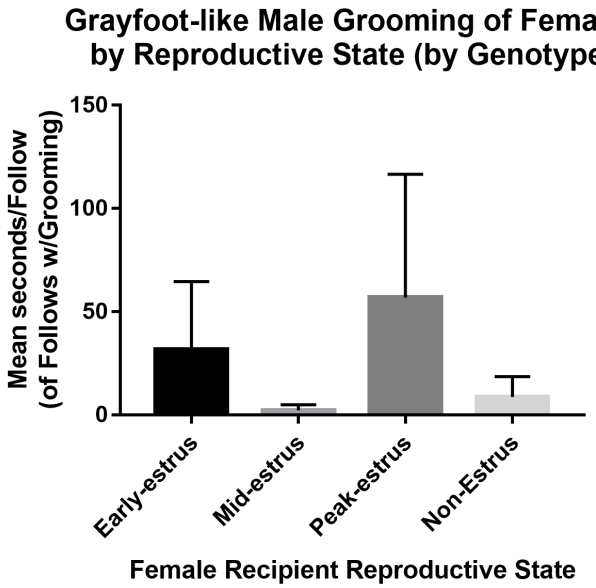


Figure 32: Grayfoot-like (Gg) male grooming of females by reproductive state

Overall, this hybrid group could be characterized as having Grayfoot-like grooming behavior, as (1) the group was not observed to groom often, (2) males in this group did not groom females more than females groomed males, and (3) when males groomed females, it was primarily when females were in estrus. **Prediction 2 was not supported**, as Kinda-like males were not observed to groom across all reproductive conditions. Rather, they groomed estrous females significantly more than non-estrous females (Figure 27 and Figure 30). Conversely, **Prediction 3 was supported** as Grayfoot-like males groomed estrous females significantly more than non-estrous females (Figure 27 and Figure 30).

Grooming and Ancestry (Prediction 4)

In order to assess whether ancestry, as determined by genotype or phenotype, influences male grooming of females, I compared male grooming of females by phenotypic ancestry (Figure 33) and by genetic ancestry (Figure 34). In both cases, the Kruskal-Wallis test showed significant differences among the three groupings (Phenotype: $p<0.0001$ and Genotype: $p<0.0001$). **Prediction 4 was supported**, as Kinda-like males groomed females significantly more than Grayfoot-like males (Phenotype: $p<0.0001$; Genotype: $p<0.0001$) and significantly more than males of intermediate ancestry (Phenotype: $p = 0.015$; Genotype: $p<0.0001$), even after carrying out post-hoc tests and correcting for multiple comparisons (Figure 33 and Figure 34).

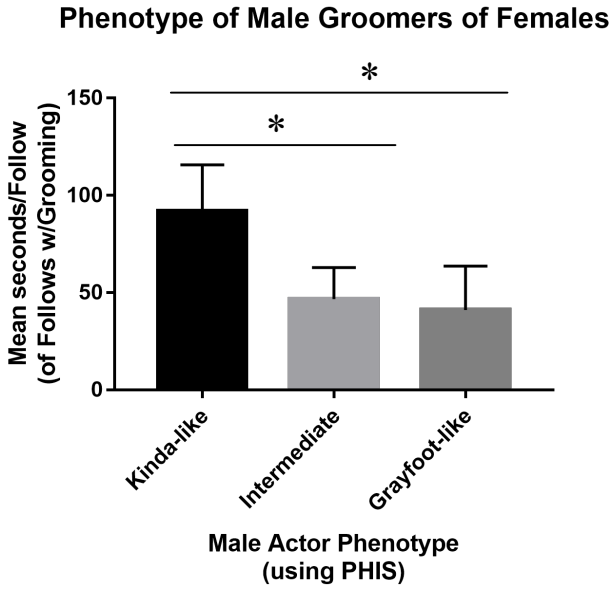


Figure 33: Phenotypic ancestry of male groomers of females

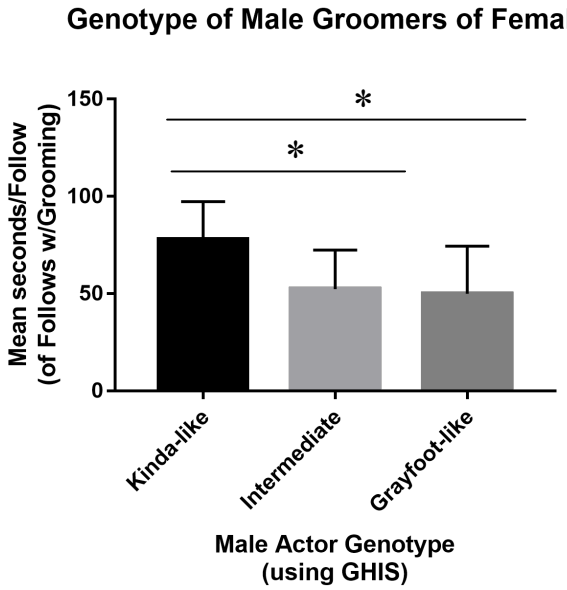


Figure 34: Genetic ancestry of male groomers of females

Further exploration of these data reveal that Kinda-like males showed little preference for grooming females of a particular ancestry (PHIS, GHIS), while Grayfoot-like males seem to have a preference for Grayfoot-like females (or Intermediate females) rather than Kinda-like females (Figure 35 and Figure 36).

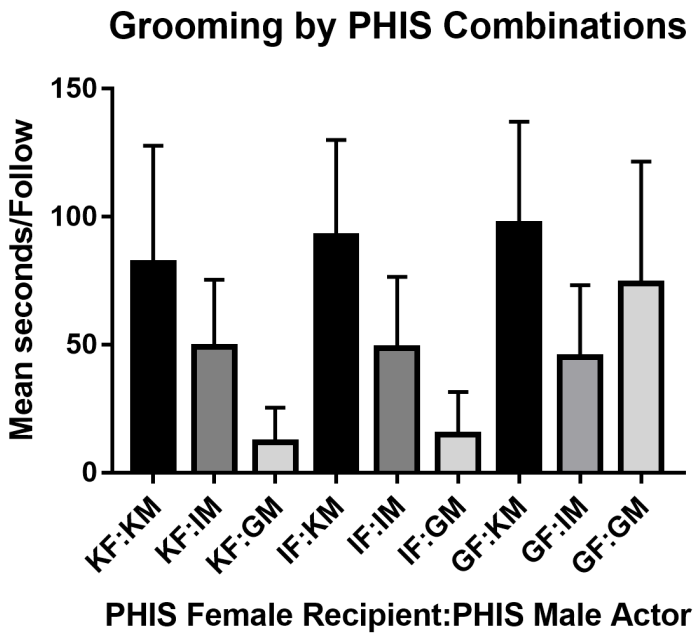


Figure 35: Phenotypic ancestry of male-female grooming combinations

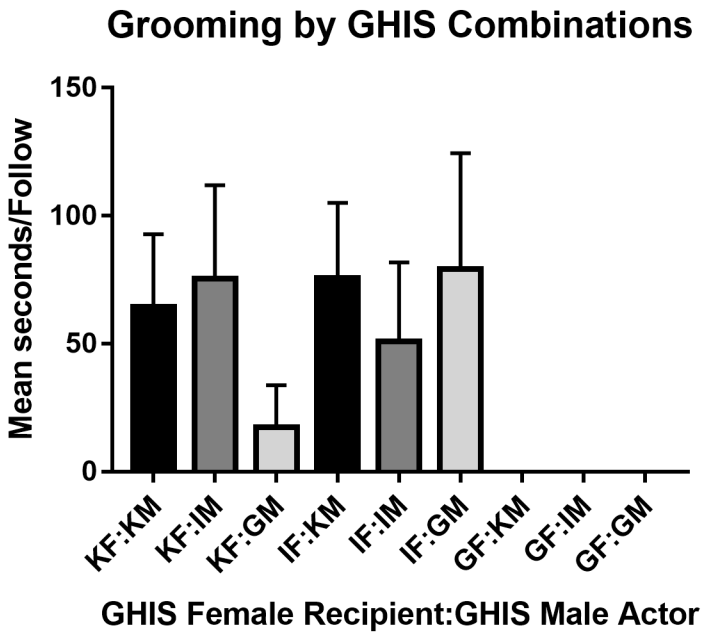


Figure 36: Genetic ancestry of male-female grooming combinations

Grooming and Mating Success (Prediction 5)

Prediction 5 was supported, as the amount of time males spent grooming females was significantly positively correlated with mating success (an indirect measure of reproductive success) (Spearman's correlation: $p<0.0001$, $R^2 = 0.66$) (Figure 37). While this is true, several of the points that account for this significant relationship are male-female dyads involving the dominant male. Since dominance rank is confounded with grooming, it is unclear which of the two variables is more influential. The potential role of dominance rank will be explored later in this chapter.

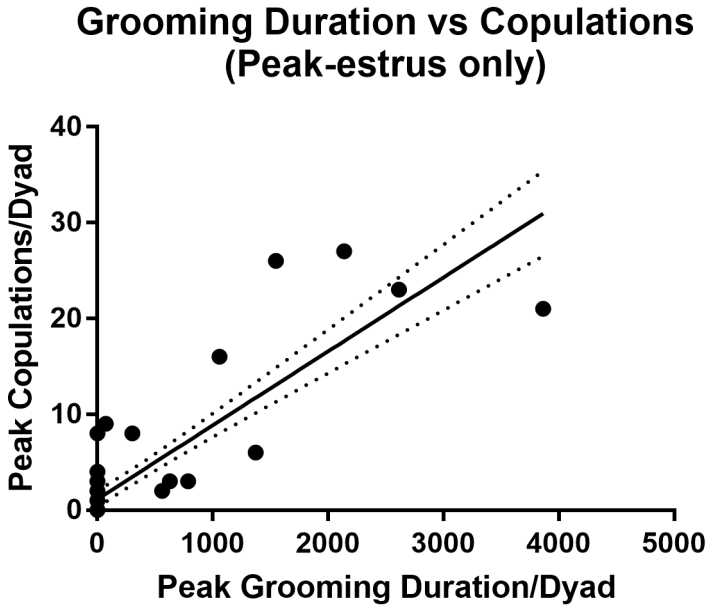


Figure 37: Relationship between grooming and reproductive success (dotted line shows 95% confidence interval)

Mating Success and Ancestry (Predictions 6)

I assessed both the phenotypic (Figure 38) and genetic (Figure 39) ancestry of males who copulated with peak estrous females, In both cases a significant difference was found in the number of copulations observed in Kinda-like, Intermediate and Grayfoot-like males (Phenotype: Kruskal Wallis test: $p<0.0001$ and Genotype: Kruskal Wallis test: $p<0.0001$). Males with kinda ancestry, as measured both by phenotype and genotype, copulated with peak estrus females significantly more than did Gp and Gg males (or Ip and Ig males) even after post-hoc tests and correcting for multiple comparisons (Phenotype: $p<0.0001$; Genotype: $p<0.0001$) (Figure 38 and Figure 39), **supporting Prediction 6**.

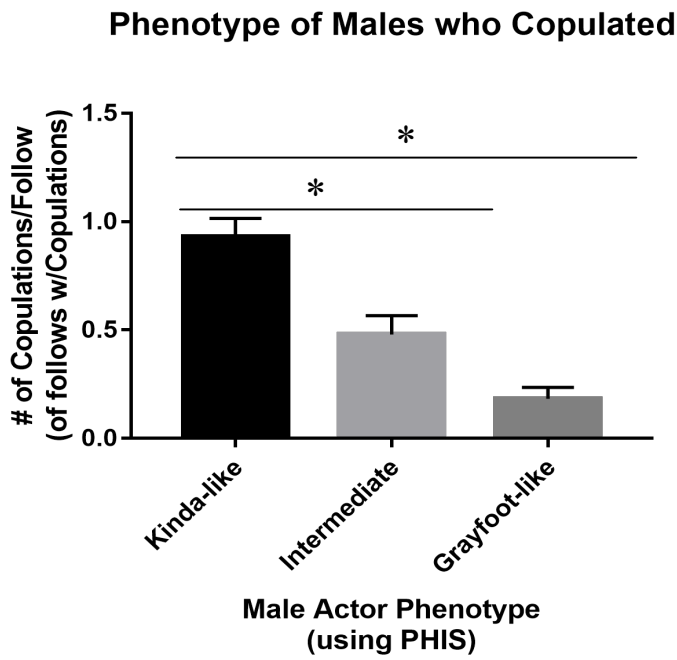


Figure 38: Mating success of males by phenotypic ancestry

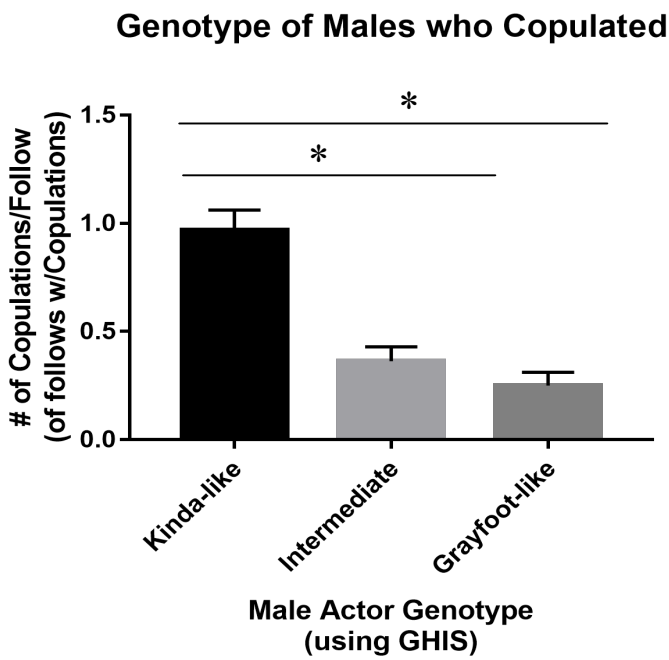


Figure 39: Mating success of males by genetic ancestry

I also investigated whether these successful males showed a preference for females of a particular ancestry. I looked at the phenotypic and genetic ancestry of the female partners with whom the males copulated. No significant difference was found between these categories for either of these measures of ancestry (Phenotype: Kruskal-Wallis test: $p = 0.31$; Mann-Whitney U test, $p = 0.39$) (Figure 40 and Figure 41).

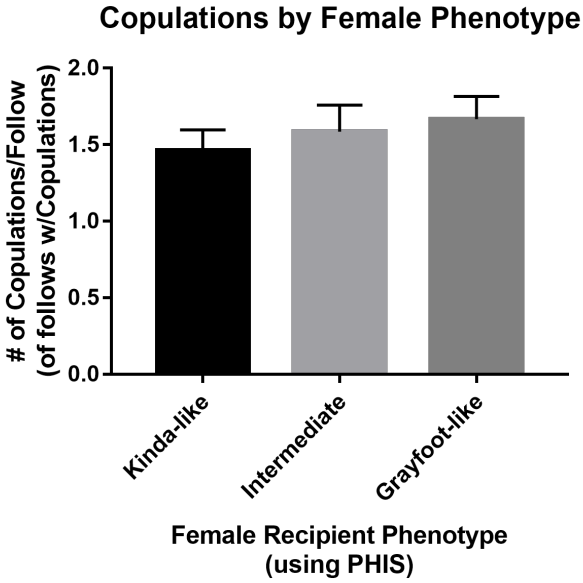


Figure 40: Phenotypic ancestry of females with whom males mated

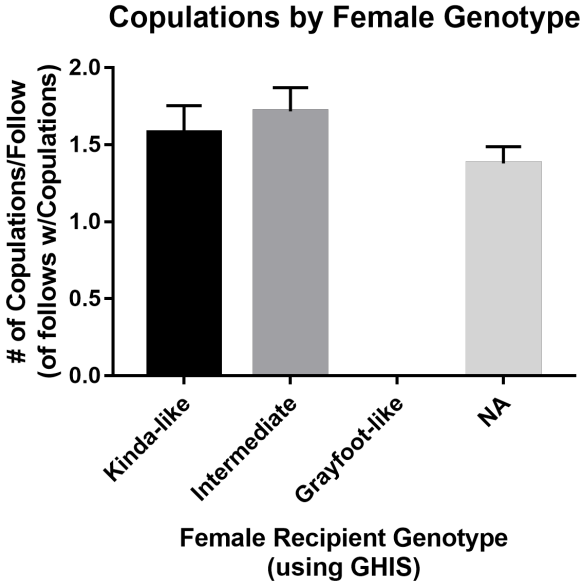


Figure 41: Genetic ancestry of females with whom males mated

Paternity and Genetic Ancestry (Prediction 7)

Kg males (GHIS < 0.10) fathered the majority of offspring (n=9 out of 13) (Table 43). The remaining fathers were Gg (n=4) (GHIS > 0.85); no Ig males fathered any offspring. The continuous data indicated a trend of males with extreme GHIS fathering more infants than those with intermediate scores (quadratic, $R^2 = 0.42$, $p = 0.07$) (Figure 42). The categorical paternity data showed that no Ig males fathered offspring; Gg males fathered as many infants as predicted, and Kg males fathered more infants than predicted (Table 43).

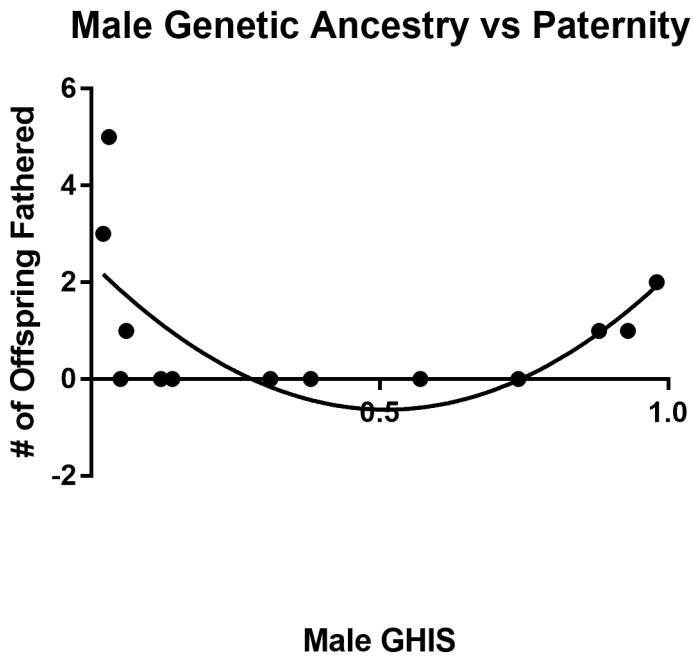


Figure 42: Male genetic ancestry versus paternity

Table 43: Offspring fathered by each genetic ancestral category (observed versus expected)

Male GHIS	Observed	Expected	Observed %	Expected %
Kinda-like (Kg) (n=6)	9	5.6	69%	43%
Intermediate (Ig) (n=3)	0	2.8	0%	21%
Grayfoot-like (Gg) (n=5)	4	4.6	31%	36%
TOTAL	**13**	**13.0**	**100%**	**100%**

Paternity and Phenotypic Ancestry (Prediction 7)

When looking at phenotypic (rather than genotypic) ancestry, PHIS for both females and males in the group ranged across the spectrum and at least one male of each phenotypic category fathered an infant (Table 44). The continuous data showed no relationship between the number of offspring fathered and male phenotype (Figure 43). Table 44 shows the observed paternities by phenotypic category compared to the expectations under random mating after taking the number of individuals in each category into account. The number of observed offspring fathered by Ip males is far greater than expected, whereas the number of Kp males fathering an infant is far less.

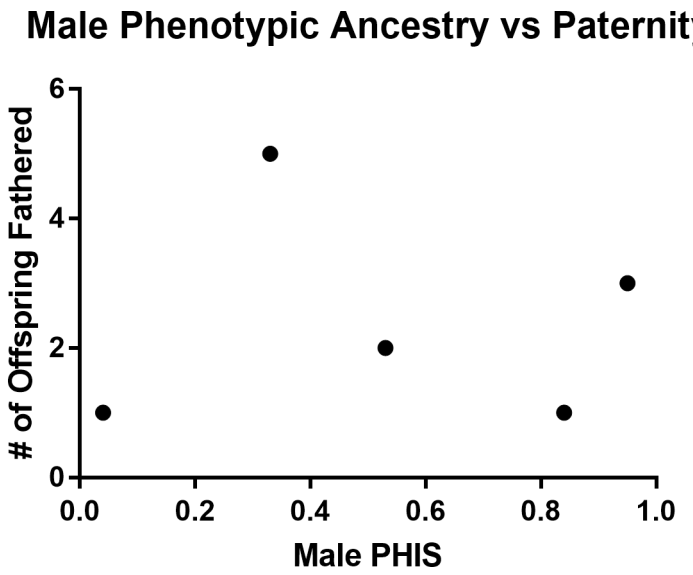


Figure 43: Male phenotypic ancestry versus paternity

Table 44: Offspring fathered by phenotypic ancestral category (observed versus expected)

Male PHIS	Observed	Expected	Observed %	Expected %
Kinda-like (Kp) (n=7)	1	5.7	8%	44%
Intermediate (Ip) (n=3)	7	2.4	54%	19%
Grayfoot-like (Gp) (n=6)	5	4.9	38%	38%
TOTAL	**13**	**13.0**	**100%**	**100%**

Overall, **Prediction 7 was inconclusive** due to the lack of correlation between genotype and phenotype. While as predicted, Kg males fathered more offspring than did Gg males (e.g. 9 out of 11 infants were sired by Kg males), Kp males actually fathered the least, rather than the most offspring (Kp males only fathered one).

7.4.2 Predictions Relating to Subhypothesis B

Prediction and Summary		Support
Prediction 8	Mate guarding correlated with mating success	Supported
Prediction 9	Dominance rank is correlated with mating success	Not supported
Prediction 10	Dominant male has the highest mating success	Supported
Prediction 11	Dominant male engaged in more reproductive-related behaviors than other males	Supported
Prediction 12	Dominant male fathered the most offspring	Supported

Mate Guarding and Ancestry

When assessing whether ancestry, as determined by genotype or phenotype, influences the mate guarding of females, I found that Ip males mate guard estrous females (early, middle, and peak estrus) significantly more than do Kp or Gp males (Kruskal-Wallis test: $p<0.0001$), even after post-hoc tests and correcting for multiple comparisons (Figure 44).

Conversely, Kg males mate guard females significantly more than do Ig or Gg males (Kruskal-Wallis: $p<0.0001$), even after post-hoc tests and correcting for multiple comparisons (Figure 45). Thus, while Kg males mate guarded females more than did Gg males, Kp males did not mate guard more than did Gp or Ip males.

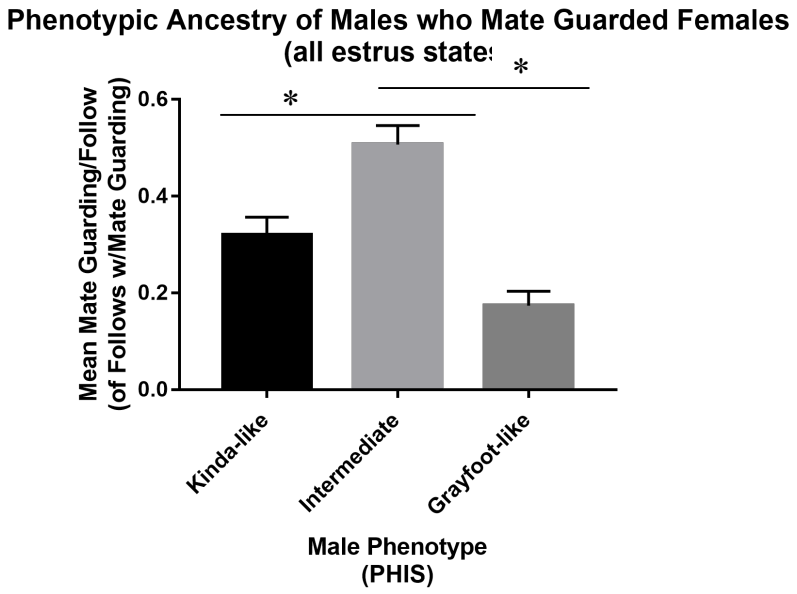


Figure 44: Phenotypic ancestry of males that mate guarded females of all estrus states

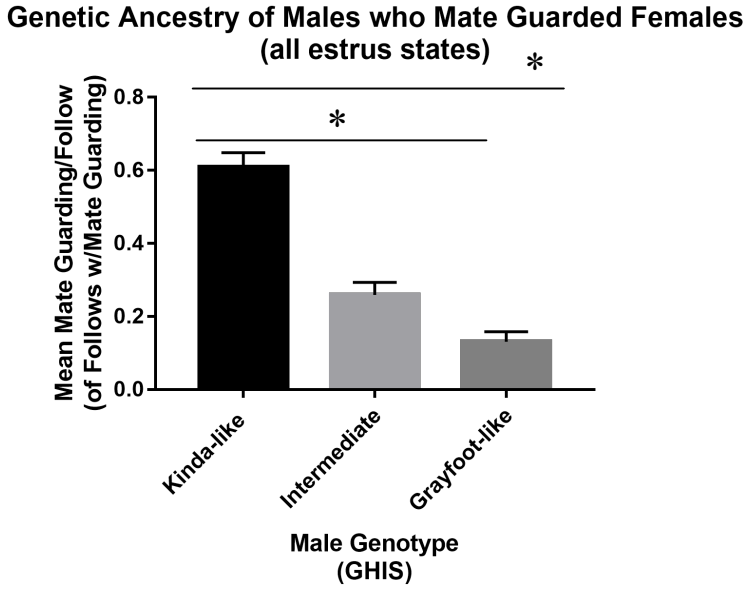


Figure 45: Genetic ancestry of males that mate guarded females in all estrus states

Mate guarding and Mating Success (Prediction 8)

Prediction 8 is supported in this group, as male mate guarding was found to be significantly positively correlated with mating success for 64 male/female dyads (Spearman's correlation: $p<0.0001$, $R^2 = 0.70$) (Figure 46). Nonetheless, several of the points that account for this significant relationship are dyads involving the dominant male. Since dominance rank is then confounded with mate guarding, it is unclear which of the two variables is more influential. As mentioned above the role of dominance rank will be investigated next.

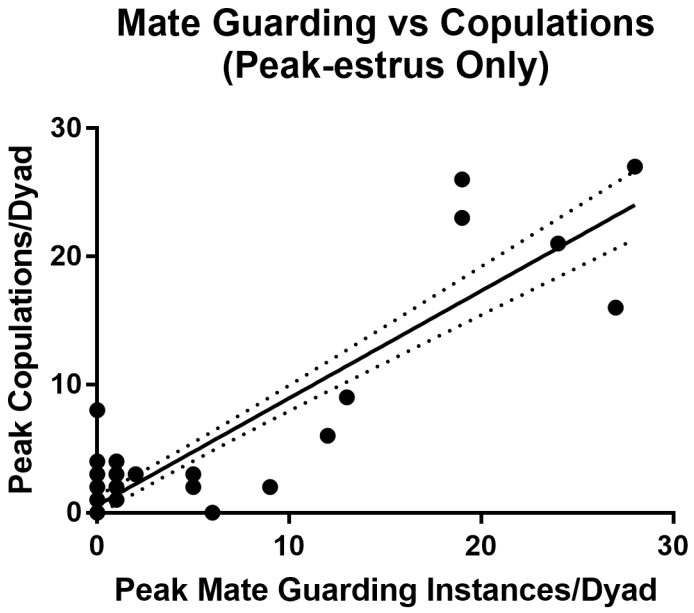


Figure 46: Relationship between mate guarding and mating success (dotted line shows 95% confidence interval)

Male Dominance Rank

In this group, male dominance rank was calculated using displacement behaviors (when one male enters the space of another causing the first male to move) and by calculating both a Normalized David's score [de Vries et al., 2006] (Figure 47 and Table 45) and Normalized Elo Rating score as described in Chapter 6 (Figure 48 and Table 45).

Table 45: Raw and Normalized Elo Rating and David's Score Dominance Rankings

Male Name	Raw Elo Rating	Normalized Elo Rating	Raw David's Score	Normalized David's Score
Strider	1563	1.00	9.45	1.00
Yogi	1290	0.73	7.18	0.56
Big K	1190	0.63	7.25	0.57
Kuyipa	1103	0.54	7.71	0.66
Jack	1062	0.50	6.30	0.39
R. Simmons	1034	0.48	7.18	0.56
Spock	1018	0.46	6.05	0.34
Solomon	988	0.43	6.86	0.50
Kink	974	0.42	6.11	0.35
Uno	933	0.38	5.35	0.21
Mavuto	846	0.29	6.80	0.49
Chifupi	739	0.18	6.04	0.34
Sub Male	706	0.15	4.46	0.04
Valentino	554	0.00	4.27	0.00

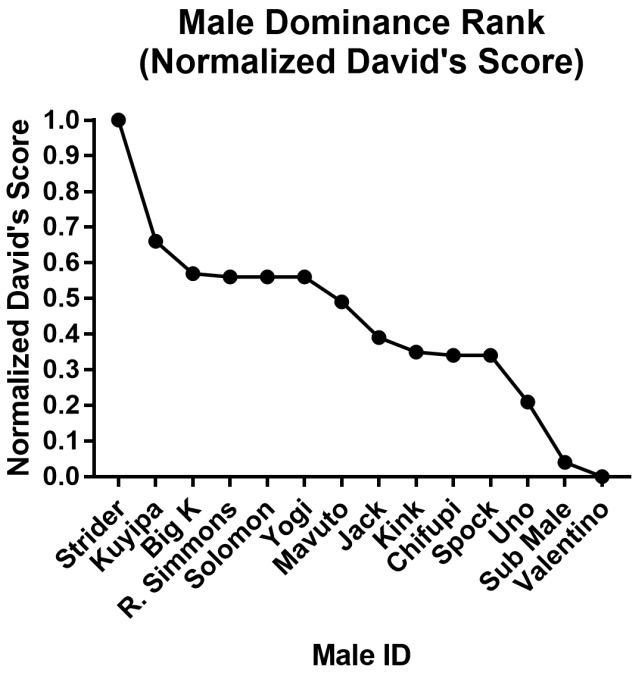


Figure 47: Male dominance rank via Normalized David's Score

Both measures resulted in linear dominance hierarchies (Normalized David's Score: R^2 = 0.894; Normalized Elo Rating: R^2 = 0.906). Also, using both measures, Strider stood out as the dominant male in the group and the subadult male and Valentino remained the lowest ranking individuals in the group (Figure 47 and Figure 48). Figure 49 illustrates how the Elo Rating method further breaks down these data to show how dominance rank varied with time, in this case month by month. However, since my data collection period was only 6 months and I was not concerned with dominance change from month to month, **I used the Normalized David's Score results in all remaining analyses.**

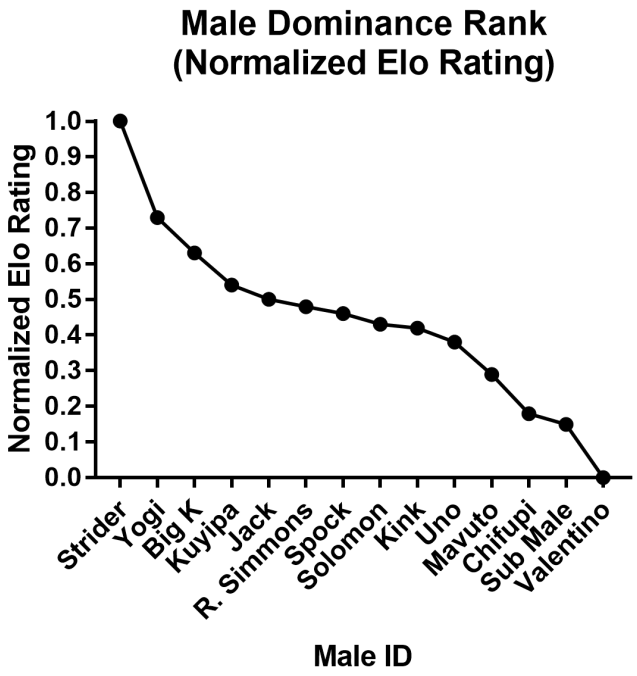


Figure 48: Male dominance rank via Normalized Elo Rating

As expected, the dominance structure of these males is linear. However, the slope of this hierarchy is not very steep, and the middle-ranking individuals are similar in rank, leading to differing results for these mid-ranking individuals across methods. On the other hand, the ranking of the dominant male and the lowest ranking males was consistent across methods. This dominance structure seems similar to that observed in grayfooted chacma baboons; however there are no data for kinda baboons with which to compare these findings.

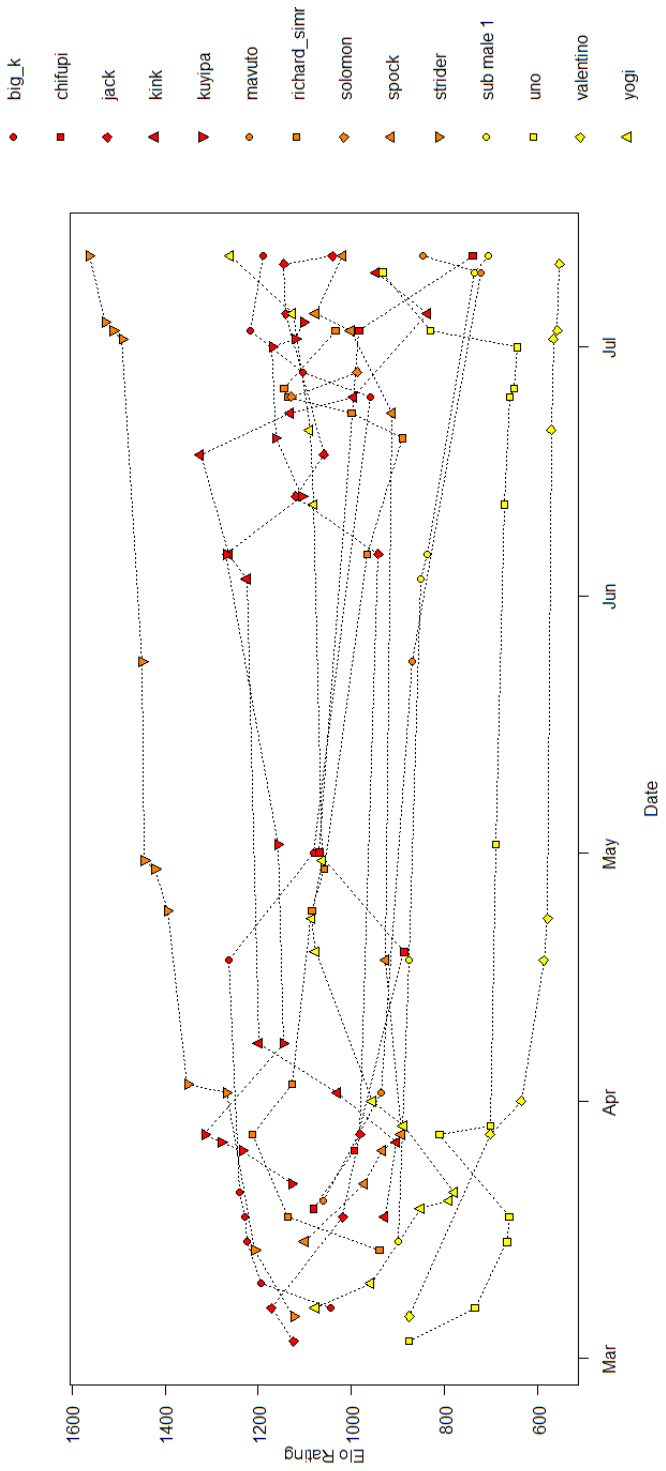


Figure 49: Male dominance rank (by month) using Elo Rating method. Each male is shown with a different color. All males begin with an equal score; then their Elo Rating changes for each sequential win or loss over time.

Dominance Rank and Mating Success (Prediction 9 and 10)

I assessed the relationship between mating success (i.e. number of copulations with peak estrous females) and dominance rank (Figure 50). **Prediction 9 was not supported**, as no positive linear correlation exists between male dominance rank and mating success (Spearman's correlation: $p = 0.29$; $R^2 = -0.313$). As dominance rank rises, mating success in the group does not necessarily also increase.

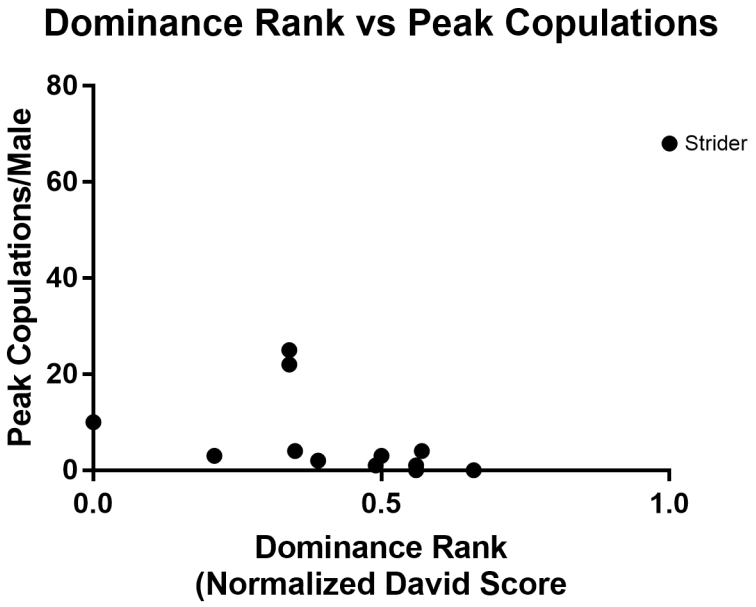


Figure 50: Relationship between male dominance rank and mating success

The dominant male (Strider) in the upper right hand corner of Figure 50, however, is an outlier and his number of copulations with peak estrus females (n = 68) far surpassed all of the other males in the group. This can be further observed in Figure 51, where I assessed each male's total number of copulations broken down by the female recipient's estrus state. The dominant male, Strider, had the greatest number of overall copulations, as well as the greatest number of copulations with both peak and mid estrous females (Figure 51 and Table 46). Thus **Prediction 10 was supported,** as the dominant male had the highest mating success in this group.

Table 46: Number of copulations by male with females of various estrus states; note that the Subadult Males category includes more than one male.

Male ID	Early Estrus	Mid Estrus	Peak Estrus	Total
Big K	59	29	5	**93**
Chifupi	34	31	27	**92**
Jack	20	1	2	**23**
Kink	6	0	5	**11**
Kuyipa	4	0	0	**4**
Mavuto	3	0	1	**4**
R. Simmons	16	0	0	**16**
Solomon	7	5	7	**19**
Spock	4	18	24	**46**
Strider	22	129	77	**228**
Subadult Males	127	60	26	**213**
Uno	4	1	3	**8**
Valentino	32	18	10	**60**
Yogi	8	2	1	**11**
Total	**346**	**294**	**188**	**828**

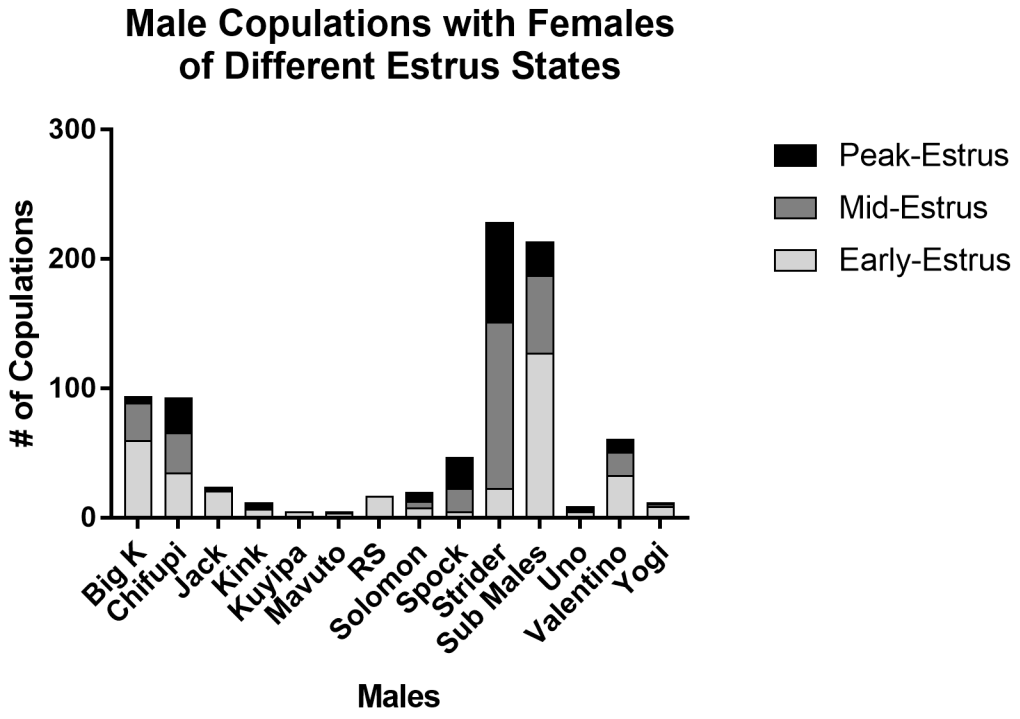


Figure 51: Total Copulations by male with females in various estrus states; note that the category "Sub Males" includes more than one subadult male.

Reproductive-related behaviors by Individual Male (Prediction 11)

I looked at the individual variation in the reproductive-related behaviors of grooming and mate guarding. The dominant male (Strider) did the most grooming (Figure 52) and also the majority of the mate guarding (Figure 53).

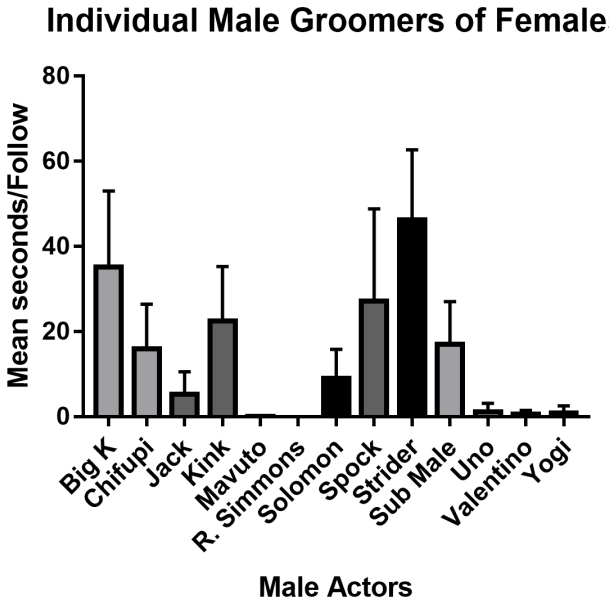


Figure 52: Male:Female Grooming by Individual; dominant male groomed females the most.

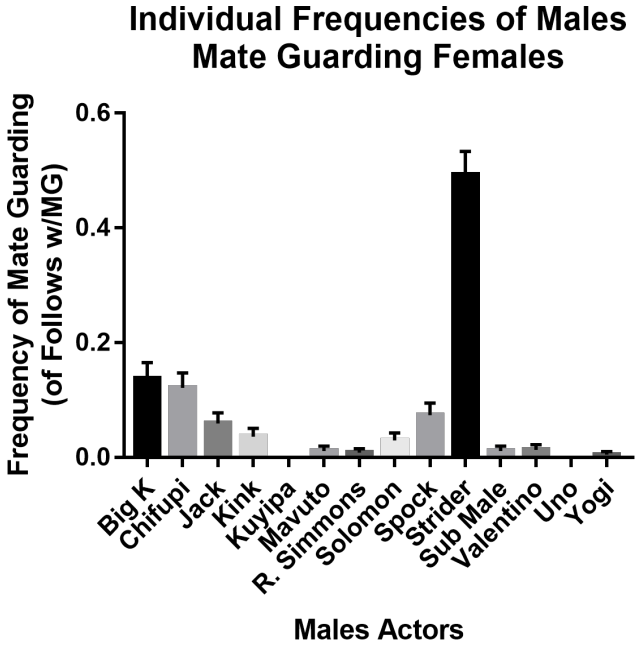


Figure 53: Frequency of mate guarding per male; the dominant male, Strider, mate guarded females the most.

Figure 54 breaks down the mate guarding data further to show the frequency of male mate guarding of females of different estrus states. While there was no significant difference between the overall amount of time males spent mate guarding early, middle, and peak estrous females ($p = 0.0784$) (Figure 54), the individuals that participated in mate guarding during each of these estrous stages clearly varied.

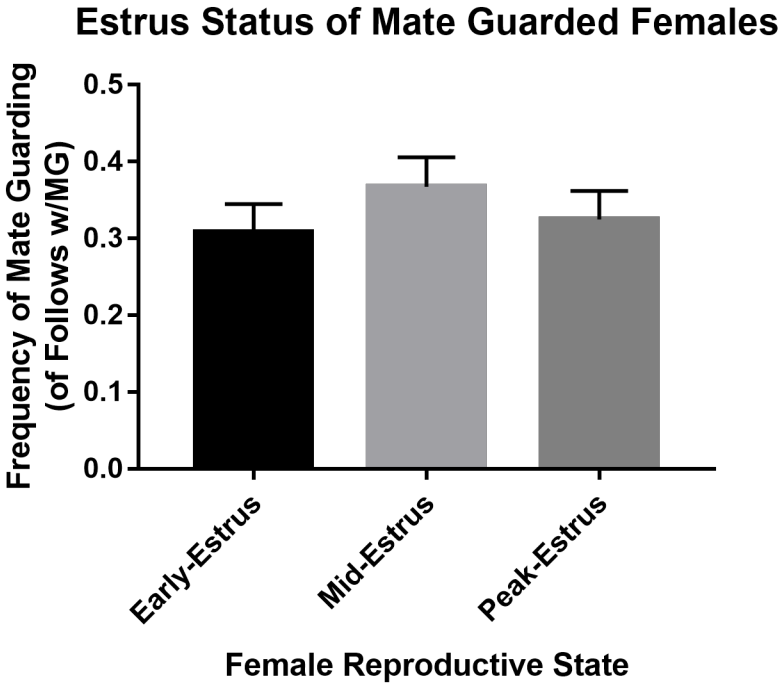


Figure 54: Male mate guarding of females of different estrus states

Since male mate guarding of peak estrous females is likely to be a better measure of mating success than total mate guarding or mate guarding of females of other reproductive states, I assessed the number of instances of mate guarding with peak estrous females by male (Figure

55). This figure illustrates that the mate guarding of peak estrous females is similarly or even more skewed in favor of the dominant male than are the overall mate guarding results (Figure 53).

These results **support Prediction 11**, as the dominant male in the group engaged in the most reproductive-related behaviors of any male in the group. He was observed to both groom and mate guard females more, particularly when females were peak estrus.

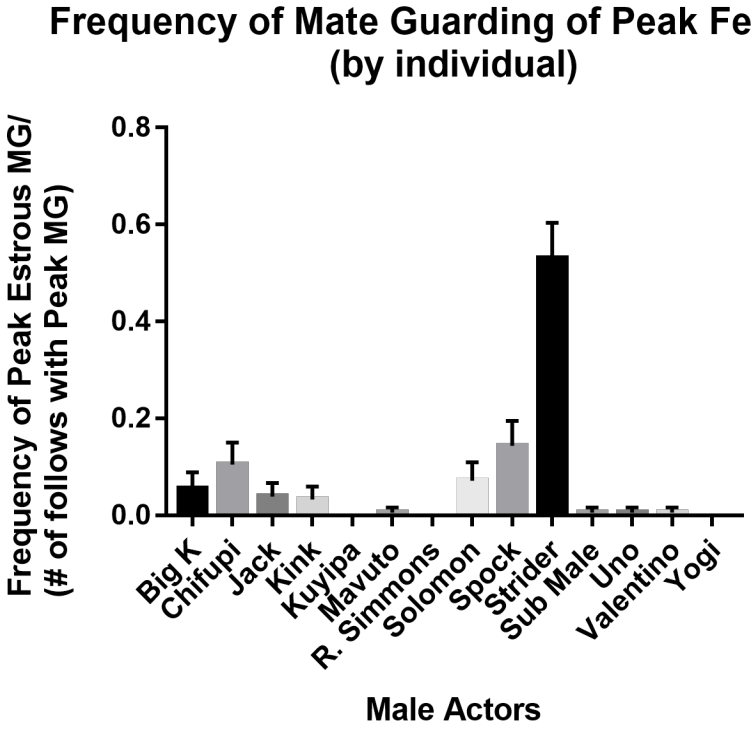


Figure 55: Mate guarding of peak estrus females by male

Paternity by Individual (Prediction 12)

Prediction 12 was supported, as Strider, the dominant male, fathered the most offspring of any of the males in the group, having fathered 5 out of 13 infants in the group. However, since the paternity data are temporally incongruent with the behavioral data, it cannot be known whether or not Strider was the dominant male at the time he mated with these females.

Also, while Strider fathered the most offspring of any male in the group, he was not the only father in the group (Solomon = 3, R. Simmons = 2, Kink = 1, Jack = 1, Spock = 1). Thus, no single male had exclusive reproductive success within the group. As the dominance rank of all males during the time of conception is unknown it is impossible to know the relationship between their dominance ranks and paternity.

Male Dominance Rank and Ancestry

Overall, no linear correlation exists between male dominance rank and ancestry, using either phenotypic or genetic measures of ancestry (Spearman's correlation: PHIS: r = 0.5238, p=0.06; GHIS: r = -0.2351, p=0.4365) (Figure 56 and Figure 57). The dominant male's phenotype and genotype are not concordant. His phenotype is Intermediate (Ip) (PHIS = 0.33), but his genotype is far towards the kinda end of the genetic continuum (GHIS = 0.03) (Table 39).

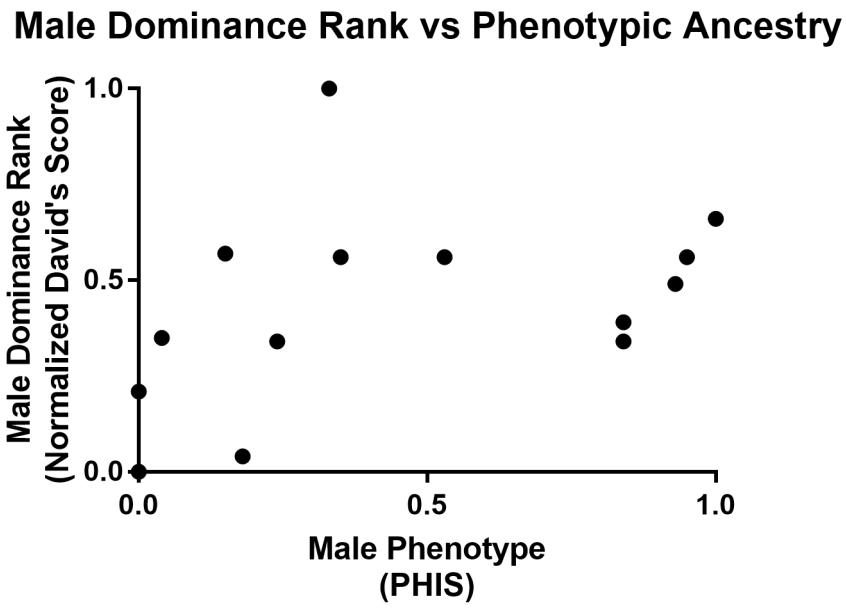


Figure 56: Male dominance rank versus male phenotypic ancestry

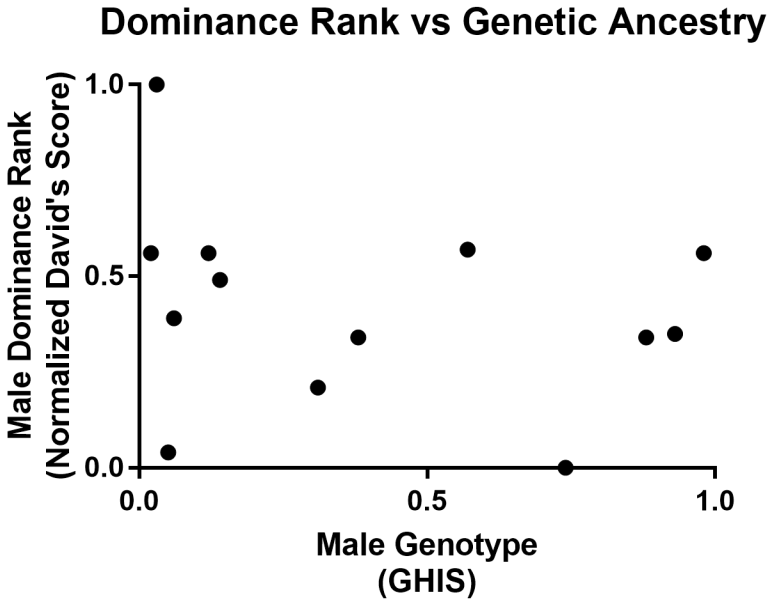


Figure 57: Male dominance rank versus male genetic ancestry

7.4.3 Predictions Relating to Hypothesis 2

Prediction and Summary		Support
Prediction 13	Mother-father PHIS are significantly correlated	Not supported
Prediction 14	Mother- father GHIS are significantly correlated	Supported

In order to investigate whether successful mating is a function of phenotypic similarity, I looked at the relationship between the PHIS of mother-father pairs (Prediction 13) (Figure 58). No significant correlation was found (Pearson's correlation: $p=0.21$). Males and females of all phenotypic combinations resulted in offspring. Ip males were the most reproductively successful; however this was due to the contribution of only 2 males.

In addition, Table 47 shows the observed offspring by phenotypic parental ancestry compared to expectation under random mating after taking the number of individuals in each category into account. This table reveals that Ip males were more reproductively successful with all females than expected, the observed numbers of offspring produced from phenotypic combinations with Gp males was similar to expectations, and the number of parental combinations involving Kp males was far less than expected. Out of 7 Kp males, only a single male fathered an offspring and it was with a Gp female.

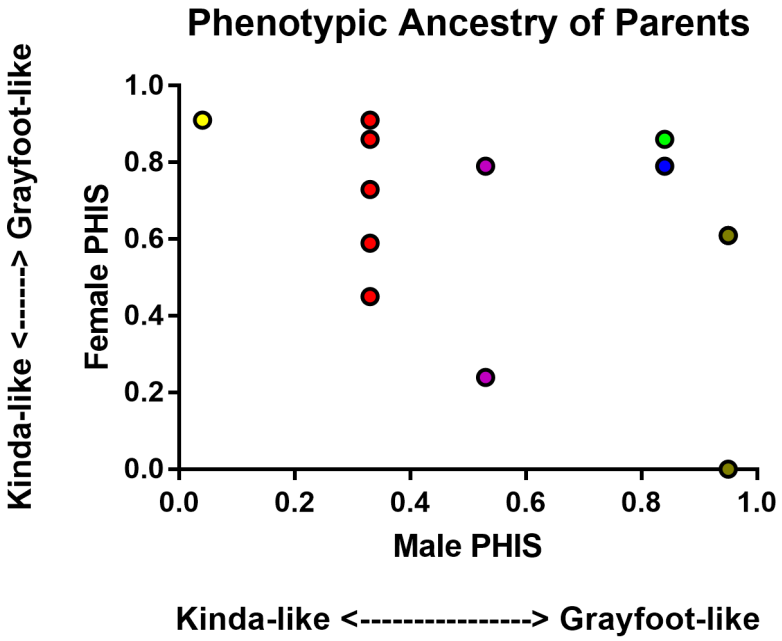


Figure 58: Phenotypic Ancestry of Parents; each dot represents an infant; each color represents a different male: red = Strider; brown = Solomon; green = Jack; yellow = Kink; blue = Spock; purple = R. Simmons.

Table 47: Offspring by each parent's phenotypic ancestral combination (observed versus expected); K = Kinda-like, I = Intermediate, G = Grayfoot-like

Father/Mother PHIS Category	Observed	Expected	Observed %	Expected %
Kp/Kp	0	1.2	0%	9.2%
Kp/Ip	0	1.2	0%	9.2%
Kp/Gp	1	3.3	8%	25.3%
Ip/Kp	2	0.5	15%	3.9%
Ip/Ip	1	0.5	8%	3.9%
Ip/Gp	4	1.4	31%	10.9%
Gp/Kp	1	1.0	8%	7.9%
Gp/Ip	2	1.0	15%	7.9%
Gp/Gp	2	2.8	15%	21.7%
TOTAL	**13**	**13.0**	**100%**	**100%**

The distribution of the observed results was not significantly different from what would be expected by chance. The number of offspring born to fathers of each phenotypic category was not dependent upon female phenotype (Fisher Exact Test; p = 0.79) (Table 48). These findings reveal that **Prediction 13 is not supported** as successful reproduction does not appear to be a function of phenotypic similarity.

Table 48: Number of offspring born to each parental PHIS category

	Female PHIS			
Male PHIS	Kinda-like (Kp)	Intermediate (Ip)	Grayfoot-like (Gp)	**Grand Total**
Kinda-like (Kp)	0	0	1	**1**
Intermediate (Ip)	2	1	4	**7**
Grayfoot-like (Gp)	1	2	2	**5**
Grand Total	**3**	**3**	**7**	**13**

GHIS of Mother-Father Pairs (Prediction 14)

In order to investigate whether successful mating is a function of genetic similarity (as measured by GHIS), I looked at the relationship between mother-father GHIS scores (Figure 59). A significant correlation was found (Pearson's correlation: p=0.008) illustrating that Gg males primarily fathered offspring with females towards the grayfoot end of the spectrum while Kg males primarily fathered offspring with females at the kinda end of the spectrum (Figure 59). Table 49 also shows that Kg males mated with Kg and Ig females more than expected by chance, as did Gg males with Gg and Ig females. All other combinations produced no offspring. For the 13 offspring in which both parents are known, 6 have parents who are both Kg and 2 have parents that are both Gg. In the 5 remaining cases where the parents are not of the same category, 3 of them involve a strongly Kg male mating with an Ig female and 2 of them involve a strongly

Gg male mating with an Ig female, resulting in infant GHIS that are either high or low. No offspring had parents that had GHIS from opposite extremes (e.g. either Kg father x Gg mother or Gg father x Kg mother) (Table 49). This pattern suggests assortative mating.

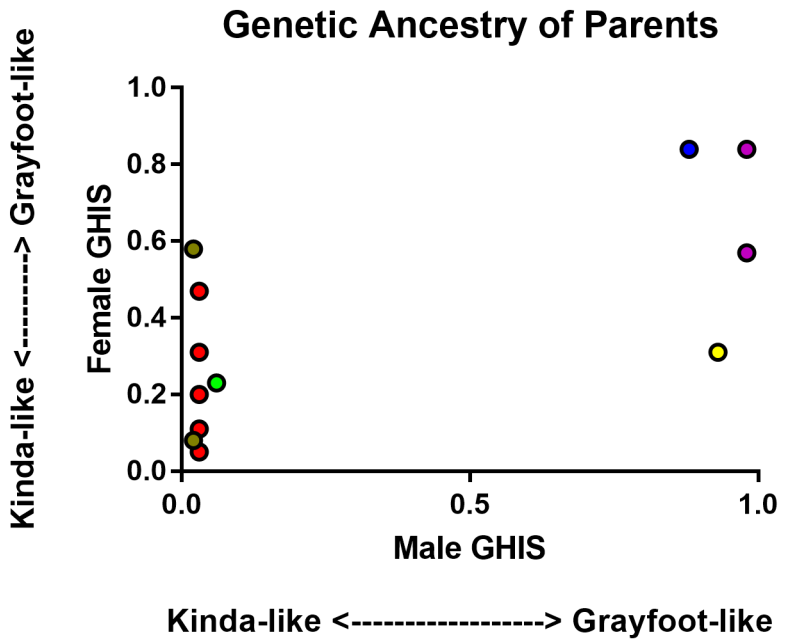


Figure 59: Genetic Ancestry of Parents; each dot represents an infant; each color represents an different male: red = Strider; brown = Solomon; green = Jack; yellow = Kink; blue = Spock; purple = R. Simmons.

Table 49: Offspring by each parent's genetic ancestral combination (observed versus expected); K = Kinda-like, I = Intermediate, G = Grayfoot-like

Father/Mother GHIS Category	Observed	Expected	Observed %	Expected %
Kg/Kg	6	3.1	46%	24.1%
Kg/Ig	3	2.1	23%	16.1%
Kg/Gg	0	0.3	0%	2.7%
Ig/Kg	0	1.6	0%	12.1%
Ig/Ig	0	1.0	0%	8.0%
Ig/Gg	0	0.2	0%	1.3%
Gg/Kg	0	2.6	0%	20.1%
Gg/Ig	2	1.7	15%	13.4%
Gg/Gg	2	0.3	15%	2.2%
TOTAL	**13**	**13.0**	**100%**	**100%**

The distribution of the observed results was significantly different from what would be expected by chance. The number of offspring born to fathers of each phenotypic category was dependent upon female phenotype (Fisher Exact Test; $p = 0.02$) (Table 50). This result seemed to be attributed to differences in offspring output between Kg and Gg males ($p = 0.036$), however, after correcting for multiple comparisons, the results were no longer significant. Overall, these findings **support Prediction 14,** as successful reproduction appears to be a result of parental genetic similarity.

Table 50: Number of offspring born to each parental GHIS category

	Female GHIS			
Male GHIS	Kinda-like (Kg)	Intermediate (Ig)	Grayfoot-like (Gg)	**Grand Total**
Kinda-like (Kg)	6	3	0	**9**
Intermediate (Ig)	0	0	0	**0**
Grayfoot-like (Gg)	0	2	2	**4**
Grand Total	**6**	**5**	**2**	**13**

7.4.4 Generalized Linear Mixed Model (GLMM)

Using the methods described in Chapter 6 (page 129), I created a Generalized Linear Mixed Model (GLMM) to ascertain the relative importance of each behavioral, phenotypic, or genetic factor and how together these factors influence male mating success. The AIC for the final GLMM model chosen was 210.3. This model revealed that male dominance rank ($p<0.001$), mate guarding of peak estrus females ($p<0.0001$), and male genotype ($p<0.05$) were significant factors in male mating success in this group (Table 51). Grooming, while significantly positively correlated with mating success (Figure 37), was not a significant factor in the GLMM model ($p=0.39675$) (Table 51). This is likely due to the fact that the mate guarding and grooming variables are correlated with one another, yet much more mate guarding was seen than grooming behavior.

I also ran the same model with the grooming variable removed. While the AIC score was lower (AIC = 209), it was not significant, and the results were otherwise the same. I therefore decided to keep grooming in the final model.

Table 51: Results from the GLMM model

Fixed Factors	Estimate	Std. Error	z value	Pr(>|z|)	Sig.
(Intercept)	1.547008	0.901868	1.715	0.08628	NS
Peak Follows (Foll_Peak)	0.013103	0.008168	1.604	0.10866	NS
Dominance Rank (M_Rank_DS)	-0.376837	0.118909	-3.169	0.00153	<0.001
Peak Mate Guarding (MG_Peak)	0.166917	0.033084	5.045	4.53e-07	<0.0001
M:F Grooming (GrmMin_ALL)	0.010946	0.012917	0.847	0.39675	NS
Male Phenotype (M_PHIS)	-0.642409	0.545657	-1.177	0.23907	NS
Male Genotype (M_GHIS)	-1.049904	0.471338	-2.227	0.02591	<0.05

Chapter 8: Discussion

In this chapter, I place the results from Chapter 7 (page 134) in the context of each hypothesis and prediction. For a review of the terminology related to the individual's phenotypic and genotypic categories see page 19.

Hypothesis 1: Kinda male affiliative social interactions are an adaptive reproductive strategy in hybrid zones.

Discussion

If, in the past, the observed asymmetry was a result of small kinda males gaining increased access to females relative to the large grayfoot males, species differences in reproductive-related behaviors such as grooming, mate guarding, and dominance rank may have been relevant to this noted differential in reproductive success.

Subhypothesis A: The kinda males' propensity to groom females regardless of their reproductive state may have given them an advantage in their interactions with females.

Prediction and Summary		Support
Prediction 1	Male:Female grooming > Female:Male grooming	Partial support
Prediction 2	K-like males groom across all reproductive states	Not Supported
Prediction 3	G-like males will only groom estrus females	Supported
Prediction 4	Grooming by K-like males > G-like males	Supported
Prediction 5	Male grooming is correlated with mating success	Supported
Prediction 6	Copulation rate of K-like males > G-like males	Supported
Prediction 7	K-like males have more offspring than G-like males	Inconclusive

When the two species first came into contact, the kinda males' greater propensity to groom females across all estrus states might have given kinda males an advantage in their relations with females. This could explain why grayfoot females allowed kinda males to approach and mate with them, even though kindas are small and display few outward signals of competitive ability (e.g., small canine teeth relative to grayfoots).

Overall rates of grooming in this group approximate a grayfoot rather than a kinda pattern: female grooming of males exceeds that of male grooming of females (Prediction 1) (Figure 23), and all males primarily groom estrous females (Predictions 2 and 3) (Figure 26). On the other hand, Kinda-like males groomed females significantly more than did Grayfoot-like males (Prediction 4) (Figure 33 and Figure 34) and phenotypically Kinda-like males groomed females more than Kinda-like females groomed males (Prediction 1) (Figure 24). Importantly, while Kinda-like males showed no preference for females of any particular type (Figure 35 and Figure 36), grayfoot males showed a grooming preference for females of their own kind (Figure 35 and Figure 36). The propensity to groom seems tied to increased mating success, as Kinda-like males also had greater mating success than Grayfoot-like males (Figure 38 and Figure 39), and the amount of time all males in this group spent grooming females (of all reproductive conditions) was significantly positively correlated with mating success (Prediction 5) (Figure 37). Overall these results suggest that while all males in this group seem to focus their energy on estrous females and not groom females more than the females groom them, the increased grooming by Kinda-like males in the group relative to that by Grayfoot-like males may result in females being more tolerant of Kinda-like males (or perhaps preferring them more) thereby resulting in the Kinda-like males' increased mating success.

In terms of mating behavior, the asymmetry (whereby all hybrids were the result of kinda males with grayfoot females) [Jolly et al. 2010] could have resulted from kinda males gaining increased mating access to females compared to grayfoot males, females preferring to mate with kinda males, or all males simply preferring to mate with large grayfoot females with larger sexual swellings. Regardless, if this asymmetry were at work in this group, I would expect that Kinda-like males would produce more offspring than would Grayfoot-like males.

Results from this study group suggest that male reproductive success (as measured by mating success and paternity) is indeed biased in favor of individuals having a kinda heritage. Overall, the findings suggest that Kinda-like males have greater mating success (Prediction 6) (Figure 38 and Figure 39) and Kg males are more reproductively successful than their Grayfoot-like male counterparts (Prediction 7) (Table 49). On the other hand, Kp males sired fewer offspring than Ip and Gp males (Prediction 7) (Table 47), reflecting the disproportionate success in mating of the dominant male (Kg but Ip) (Figure 50). Conversely, behavioral observations from this study group do not support the idea that at the time of initial hybridization all males simply preferred to mate with large grayfoot females, as males in this group did not show mating preference for females of any particular genotype or phenotype (Figure 40 and Figure 41), and females of all ancestries were observed to produce offspring (Figure 58 and Figure 59).

Subhypothesis B: Alternatively, since high male dominance rank and mate guarding have been associated with high male reproductive success in baboons, high dominance rank and/or increased mate guarding resulted in increased mating and reproductive success.

Prediction and Summary		Support
Prediction 8	Mate guarding correlated with mating success	Supported
Prediction 9	Dominance rank is correlated with mating success	Not supported
Prediction 10	Dominant male has the highest mating success	Supported
Prediction 11	Dominant male engaged in more reproductive-related behaviors than other males	Supported
Prediction 12	Dominant male fathered the most offspring	Supported

As has been previously observed, high male dominance rank and mate guarding are often associated with high male reproductive success in baboons [Bulger, 1993; Weingrill et al., 2000, 2003; Alberts et al., 2006]. It could be that dominance rank and/or mate guarding were more influential in increased mating and reproductive success (paternity); however if a kinda male was able to become dominant or was more effectively able to mate guard estrous females, this may have resulted in the hybrid asymmetry.

Mate guarding was significantly positively correlated with mating success, in this group (Prediction 8) (Figure 46). In terms of ancestry, Kg males mate guarded females more than did Gg males (Figure 45); however, Ip males mate guarded females more than both Kp or Gp males (Figure 44). These conflicting results can likely be explained by considering the incongruent phenotype and genotype of the dominant male in this group (Kg but Ip). Since the dominant male mate guarded females the most, it is likely his behavior that is driving these results (Figure 55).

In terms of dominance rank, while there was no linear correlation between dominance rank and mating success across all males of the group (Prediction 9), the highest-ranking male in the group (Strider) had both the highest mating success (Prediction 10) and reproductive output (Prediction 12) (Figure 50). He also engaged in the most reproductive-related behaviors (Prediction 11): he groomed females (Figure 52) and mate guarded females the most (Figure 53). These results are not surprising, as they are consistent with what is expected of dominant males in most baboon species. Notably, the dominant male in this group (Strider), was genetically kinda (although Ip) which could result in the proposed asymmetry (Figure 17 and Figure 7).

In addition, while this dominant male was only continuously observed for 6 months, other less systematic data recorded from the group between 2013 and 2015 suggest that he has been dominant for more than 2 years, significantly longer than generally seen in chacma baboons [Hamilton & Bulger, 1990; Palombit et al., 2000]. Additionally, the few times in which his dominance was contested, the male trying to obtain dominance (Solomon) was also Kg (although Gp). This observation suggests that genetically (but not necessarily phenotypically*)* Kinda-like males (Kg, but not Kp) are better able to gain alpha status than Gg males. Both of these Kg males (Strider and Solomon) fathered the greatest numbers of offspring, actively contributing to the hybrid asymmetry. These results raise the possibility that Kg males are better able to gain dominant status or are able to maintain alpha status longer than Gg males. I hope to explore this in future studies.

Hypothesis 2: Genetic or obstetrical, rather than behavioral factors, contributed to the reduced production of offspring by grayfoot male/kinda female matings.

Discussion

While we were unable to record the obstetrically relevant morphometric measures in the field, I can comment on a few observed pregnancies that did not produce viable offspring (although occurrences of this kind are likely in any baboon group). During the behavioral data collection, two females were observed to be pregnant and then either miscarry or lose their infants. However, it is unclear whether either of these cases were the result of a Gp male mating with a Kp female, as the one female who was Kp was not remarkably small and it is impossible to know the phenotype of the male with whom she conceived.

More importantly, I was able to address the issue of genetic incompatibility. No correlation was found between the PHIS of the known mothers and fathers of infants (Figure 58) suggesting that differences in phenotypic features between the two species is of little importance in determining who will be a parent (Prediction 13). These results suggest no reason to suspect that premating behavioral mechanisms are leading to this asymmetry. On the other hand, a significant correlation was found between the GHIS of the known mother-father pairs (Prediction 14) (Figure 59). In this group, offspring resulting from both Gg fathers and Kg mothers and Kg fathers and Gg mothers were absent. None of the 13 offspring was the result of parents on the genetic extremes (Table 37). This result may suggest that direct mating between Kg and Gg individuals is either undesirable or genetically less successful. The parents of the majority of

offspring in this study were a product of individuals of similar genetic ancestries mating with each other (Kg males with Kg females and Gg males with Gg females) (Table 37). This suggests a degree of assortative mating within this group. When the parents of offspring differed genetically, the offspring were a product of a Kg male and an Ig female or a Gg male with an Ig female; however there were more offspring produced from the former rather than the latter pairing. This implies that the primary vector for asymmetric hybridization in this group is the increased frequency of offspring produced by Kg males with Ip females compared to Gg males with Ig females. Similarly, when looking at the continuous GHIS data, it was evident that only males with extremely low or high GHIS were reproductively successful (i.e. Kg or Gg) (Figure 59).

These findings suggest that regardless of who mates with whom, only genetically similar females and males produced offspring in this group (Figure 59) and that while more research is needed to confirm this possibility, it may be that Ig males have reduced fertility, while females of all genetic ancestries are fertile. If this were true, it would suggest that the hybrid zone is primarily being maintained by relatively genetically "pure" males of either species producing offspring with females of more "mixed" or Intermediate ancestry.

Hypothesis 3: Grayfoot males misinterpreted kinda baboons as immature due to their small size, prompting inappropriate responses in the context of consortship and mating.

Discussion

I was unable to address this hypothesis since nearly all of the assumptions necessary to test this hypothesis were violated. This hypothesis assumes that adult male and female kinda baboons are small. In this study of a hybrid group, it was not known whether small males were actually Kp adults or were simply subadults (since we did not have information on their ages), and small males could not be assumed to be Kg (as Kg individuals were of varying phenotypes and body sizes). Similarly, since I did not know the ages of females, I could not determine whether small sexual swelling size was associated with parous Kp females, rather than simply being an artifact of age.

I did observe small males mating with estrous females. However, these males were almost certainly subadults rather than adults and they only managed to gain access to females that were early (rather than peak) estrus, which would not be expected to result in offspring or the noted hybrid asymmetry.

In terms of grayfoot males misinterpreting kinda females as immature, I observed that all males in this group typically found females with small sexual swellings to be undesirable. However, the females with these small swellings were not necessarily Kp or Kg. In addition, the size of these sexual swellings changed with age; females with these small undesirable sexual swellings were simply nulliparous females who had not yet reached full maturity. In time, their

swellings increased in size to be comparable to the swellings of other females in the group, at which point the male interest for these females also increased.

8.1 Conclusion

1. Hybridization within the kinda-grayfoot hybrid zone is asymmetrical, yet not entirely uni-directional, as was once thought. This hybrid group is relatively admixed (as is evidenced in the lack of correlation between microsatellites and external phenotype) which suggests that the zone has been in existence for several generations.

2. The proposed hybrid asymmetry was likely a result of genetically (but not necessarily phenotypically) kinda males having greater success with females (whether or not females prefer them). This topic should be explored further. However, there was no evidence to suggest that all males preferred females of grayfoot ancestry over other females.

3. Overall, this hybrid group displayed intermediate or grayfoot grooming behavior. However, grooming within the group varied as predicted by heritage (heritage deduced from both microsats and appearance) such that both phenotypically and genetically Kinda-like males groomed females more than did Grayfoot-like males.

This propensity for Kinda-like males to groom more suggests that this unique kinda grooming behavior may make kinda males more reproductively successful.

4. No infants were fathered by parents having genetic ancestries on opposite extremes (K x G or G x K), nor by Ig males. This suggests that Ig males may have reduced fertility and that the asymmetry in this group is being driven by a number of genetically Kinda-like (Kg) males being reproductively successful with highly genetically admixed (Ig) females.

5. No support was found to suggest that the asymmetry is due to grayfoot males misinterpreting kinda individuals as immature. However, this group was not optimal for testing this hypothesis. Thus, this hypothesis warrants further investigation in groups that contain individuals of "pure" ancestry, perhaps groups on either end of the hybrid zone.

6. Dominance rank, mate guarding and genetic ancestry are all important factors in male reproductive success in this group. The dominant male is a complicating factor as he was genetically Kinda-like. It could be that males that are genetically (but not necessarily phenotypically) Kinda-like have an advantage in gaining and/or maintaining dominance, thereby leading to the observed hybrid asymmetry. However, this idea necessitates further investigation.

Chapter 9: Summary and Conclusions

In this study, I used phenotypic, behavioral, and genetic data from a single hybrid baboon group in Kafue National Park, Zambia to examine hybridization between two of the most divergent baboons, the kinda baboon and the grayfooted chacma baboon, which differ markedly in body size and social behavior. The objectives of this study were to (1) explore potential behavioral underpinnings of a previously observed asymmetry (2) interpret the behavioral findings of this study in the context of the parents' degree of kinda or grayfoot ancestry and (3) assess the success of certain parental combinations in producing offspring.

9.1 Summary of Findings

9.1.1 Genetics

I extracted, amplified, and sequenced/genotyped DNA from fecal samples collected from all adult males and females in the group. I used the mtDNA d-loop (Figure 15) and Y-marker (DYs576) (Table 26) results to ascertain whether the same pattern of asymmetry as in Jolly et al. [2010] existed in this hybrid group. Four cases of bi-directional Y-mitochondrial discordance were detected (Table 42) reflecting hybridization. I used 7 microsatellite loci (Table 31) and the program STRUCTURE to estimate the allele frequencies of individuals in this hybrid group relative to individuals from each of the "pure" species to assign a Genetic Hybrid Index Score (GHIS) to each individual (Table 34 and Table 35). I also scored each adult on 8 physical

features in order to assign Phenotypic Hybrid Index Scores (PHIS) to each individual (Table 3 and Table 4). I found that GHIS and PHIS were not correlated (Figure 20 and Figure 21).

I used 9 microsatellite loci and the program CERVUS to estimate the parentage of 13 infants in the group (Table 27). A reproductive skew was found; three males in the group fathered most of the infants (Strider = 5, Solomon = 3, R. Simmons = 2), while two other males fathered one infant each and one infant was unable to be assigned paternity (Spock = 1, Jack = 1, unknown = 1) (Table 28). Only males with low or high GHIS scores were fathers (Figure 59), and no infants were the result of matings across the genetic extremes (no G x K or K x G parents) (Table 50). Instead, offspring were either the result of assortative mating or Kinda-like or Grayfoot-like males mating with Intermediate females.

9.1.2 Behavior

I analyzed behavioral data collected over a 6-month period using 10-minute continuous focal animal follows. I anticipated that grooming, a characteristic feature of kinda males, would be related to male reproductive success. As has been observed in other studies of grayfooted chacmas baboons, I found that overall in this group, grooming by males was infrequent, females groomed males more than males groomed females (Figure 23), and all males focused grooming on estrous females (Figure 26). However, grooming was positively correlated with mating success (Figure 37), and Kinda-like males groomed females more frequently than Grayfoot-like males (Figure 33 and Figure 34). Also, when inter-sex grooming was assessed by phenotype of the groomer, phenotypically Kinda-like males groomed females more than Kinda-like females

groomed males (Figure 24) and Kinda-like males tended to groom females of all genetic backgrounds, whereas Grayfoot-like males tended to concentrate grooming effort on females of their own kind (Figure 35 and Figure 36). This might explain the genetic asymmetry previously observed in Jolly et al. [2010]. Both genetically Kinda-like and Grayfoot-like males prioritized the grooming of peak estrous females (Figure 31 and Figure 32). However, males that were phenotypically Kinda-like focused grooming efforts on early estrous females (Figure 28), while phenotypically Grayfoot-like males monopolized peak estrous females and concentrated their grooming efforts on the peak estrous females (Figure 29). This suggests that, in contrast to the findings from the genetic hybrid index scores, the males that were Kinda-like **in appearance and/or size** were unable to compete with Grayfoot-like males or gain access to peak estrus females.

Since other baboon studies have found mate guarding, copulation, and male dominance rank to be indicative of reproductive success [Bulger, 1993; Weingrill et al., 2000, 2003; Alberts et al., 2006], I also examined these behaviors. Mate guarding was positively correlated with mating success (Figure 46). Genetically Kinda-like males mate guarded females more than did Grayfoot-like males (Figure 45). This may explain the previously observed genetic asymmetry. However, it was males that were Intermediate in appearance and/or size that did the most of the mate guarding (Figure 44).

I used displacement behaviors to calculate male dominance ranks. As expected, males in this group could be situated within a linear dominance hierarchy (Figure 47). Also, the highest ranking male in the group engaged in the most grooming (Figure 52) and mate guarding (Figure 55) behaviors, and also had the most mating success (copulations) (Figure 51) and reproductive

success (paternity; n = 5 out of 13). Overall, both genetically and phenotypically Kinda-like males had higher mating success (copulations with peak estrous females) than did Grayfoot-like males (Figure 38 and Figure 39). This is consistent with the previously observed asymmetry. However, it is important to note that the dominant male in the group was genetically Kinda-like, but phenotypically Intermediate (Table 39).

In summary, Kinda-like behaviors were largely correlated with mating and reproductive success in this hybrid group, but were largely driven by the result of the dominant male's genotype and phenotype. This suggests that high dominance rank is the biggest indicator of reproductive success in this group. These findings were corroborated by results from the Generalized Linear Mixed Model (GLMM) that showed that dominance rank, mate guarding, and male GHIS (being genetically more Kinda-like) were the most important factors in male mating success in this group (Table 51).

9.2 Formation and maintenance of the kinda-grayfoot hybrid zone

9.2.1 Type of hybridization

Prior work on the kinda-grayfoot hybrid zone suggested that it originated via asymmetric hybridization with kinda males more reproductively successful with grayfoot females than the reverse [Jolly et al., 2010]. I found two cases of hybridization in each direction, which indicates that, either originally or contemporarily, bi-directional mating or backcrossing must have been a part of the process [Wirtz, 1999].

9.2.2 Mechanisms of hybridization

The presence of mitochondrial haplotypes from both species in the males in this group indicates that males are the dispersing sex, and the presence of only grayfoot mitochondrial haplotypes in the females indicates female philopatry. This is consistent with previous studies of kinda and chacma dispersal patterns [Cheney et al., 2006; Burrell, 2009].The behavioral factors that allowed the small kinda males to be reproductively successful with large grayfoot females when they first came into contact remain largely unclear, although, the increased grooming behavior of Kinda-like males might be a major contributory factor.

9.2.3 Age of the hybrid zone

Reports suggest that kinda and grayfooted chacma baboons may have had the opportunity to hybridize since at least the mid-1970s [Ansell, 1960, 1978]. No morphological or phenotypic evidence suggests that hybridization between these two species occurred in and/or prior to the 1960s, as surveys of Zambian wildlife prior to 1960 reported kinda and grayfooted chacma baboons on either side of the Kafue River (kindas on the north and east side and grayfoots on the south and west side) [Ansell, 1960, 1978; Burrell, 2009]. As such, the Kafue River acted as a geographical barrier, which was eliminated in 1974 with the construction of the Itezhi-tezhi dam and bridge permitting hybridization over the past 40 years.

If this were a recent hybrid zone, it would be expected that the phenotype and genotype of individuals in the group would be correlated due to linkage disequilibrium [Bergman et al.,

2008]. The lack of correlation between phenotype and genotype in this hybrid group then suggests that this group has seen multiple generations of hybridization, enough for recombination to have interrupted this relationship [Bergman et al., 2008].

9.2.4 Maintenance of hybrid zone

In considering how this hybrid zone is being maintained, I report a complex interplay of genotype, phenotype and behavior. I found that genetically (but not necessarily phenotypically) Kinda-like hybrid males have a reproductive advantage over other males in the group. For males in most savanna baboon species (including chacmas), size confers an advantage when competing for mates [Muller & Wrangham, 2009; Pradhan & van Schaik, 2009]. The dominant male is often large, the most successful in consortship (mate guarding) and mating, and is the most reproductively successful (although the steepness of this reproductive skew varies across species) [Bulger, 1993; Weingrill et al., 2000, 2003; Alberts et al., 2006]. In addition to the proposed female preference for Kinda-like male grooming behavior, it may also be that male dominance rank and competitive advantage is size-dependent. This idea is consistent with the results of this study, as in general, smaller individuals were not reproductively successful, while medium to large individuals (particularly those which were genetically Kinda-like) were successful. The dominant male in this study group was genetically Kinda-like, phenotypically Intermediate (i.e. medium-sized) and fathered the most infants. He also engaged in the most mate guarding and grooming behaviors.

All baboon taxa hybridize where their ranges meet, and genetic evidence reveals frequent and extensive hybridization throughout the history of the genus, often resulting in nuclear genetic swamping [Newman et al., 2004; Wildman et al., 2004; Burrell, 2009; Zinner et al., 2011b]. Two currently active hybrid zones have been extensively studied: the anubis-hamadryas hybrid zone in Awash, Ethiopia and the anubis-yellow hybrid zone in Amboseli, Kenya. Some of results from this study parallel those from other baboon hybrid zones, while other results suggest complex mechanisms that may be specific to hybridization between kinda and grayfooted chacma baboons in Kafue National Park, Zambia. The next section describes these two extensively studied hybrid zones and places the results of this study in context.

9.3 Kafue Hybrid Zone versus the Other Baboon Hybrid Zones

9.3.1 Type and mechanism of hybridization

The anubis-hamadryas baboon hybrid zone in Ethiopia is thought to have originated via bi-directional hybridization as a result of group fusion [Beyene, 1993, 1998] and male migration of both species [Sugawara, 1982; Phillips-Conroy & Jolly, 1986; Phillips-Conroy et al., 1991, 1992; Nystrom, 1992]. The anubis-yellow hybrid zone in Kenya originated via asymmetrical hybridization due to the migration of anubis males into yellow groups [Samuels & Altmann, 1986]. However, genetic evidence across this zone suggests that cases of yellow males migrating into anubis groups, while rare, have also occurred [Charpentier et al., 2012].

The pattern of hybridization in the kinda-grayfoot hybrid zone is reminiscent of that found in the anubis-yellow hybrid zone in Kenya; while the kinda-grayfoot hybrid zone is primarily characterized by asymmetrical hybridization, genetic evidence from the present study reveals that bi-directional hybridization has also occurred.

9.3.2 Age of hybrid zone

Hybridization between anubis and hamadryas baboons in Awash, Ethiopia was first noted in the 1960s [Kummer & Kurt, 1963; Kummer, 1968; Nagel, 1973]. In the 1970s, observations by Kyoto University researchers documented hybrid individuals in groups that previously had none [Kawai & Sugawara, 1976; Shotake et al., 1977; Sugawara, 1979; Shotake, 1982]. Brett's expedition (1971-1973) surveyed and trapped baboons from 11 groups across the zone. Phenotype assessments of baboons across the zone revealed hybridization had increased in the interim, as evidenced by a smoother morphological cline compared to Nagel's [1973] results [Jolly & Brett, 1973; Phillips-Conroy & Jolly, 1981, 1986]. Genetic results from this expedition showed that species-specific mitochondrial haplotypes and Y-marker genotypes of individuals were associated with their corresponding species-specific phenotypes [Newman, 1997; Woolley-Barker, 1999]. However, in a later, detailed study of a single highly hybridized group (Group H) within this hybrid zone phenotype and genotype were found to be correlated in the older males, but uncorrelated among males born more recently [Bergman, 2000; Beehner, 2003; Beehner et al., 2005; Bergman et al., 2008]. This suggested that these older males represented earlier generations of hybrids in which linkage disequilibrium was still strong, whereas these younger

males signified later generations of admixture that had resulted in recombination breaking down the association between genotype and phenotype [Bergman et al., 2008].

The anubis-yellow hybrid zone in Kenya originated in the 1980s with anubis males migrating into yellow groups [Samuels et al., 1986]. While generally phenotype and genotype were correlated [Alberts & Altmann, 2001; Tung et al., 2008], some individuals that were initially classified as yellows carried a proportion of anubis genes, suggesting that using genetic microsatellite loci may provide better resolution than phenotypic features in revealing subtle levels of introgression [Tung et al., 2008].

My findings do not match those seen at Amboseli, but rather are more consistent with those observed in the Awash. Surveys of groups across the kinda-grayfoot hybrid zone suggest that species-specific haplotypes and Y-marker genotypes in this zone are associated with their species-specific phenotypes [Jolly et al., 2010]. The findings from this study then show that phenotype and genotype are not correlated within a highly hybridized group towards the middle of the zone. As was reported for the Awash group, this finding suggests that multiple generations of hybridization must have occurred in order to break down this relationship.

9.3.3 Maintenance of the hybrid zone

Phenotype and behavior were correlated in the anubis-hamadryas hybrid zone in Ethiopia. This relationship was strongest in groups primarily consisting of anubis individuals [Sugawara, 1979; Nystrom, 1992; Phillips-Conroy et al., 1992; Beyene, 1998]; however, broad studies across the zone also found that this pattern was true for groups consisting primarily of

hamadryas individuals. A few hybridized groups within the anubis-hamadryas zone (Group D and H) contained individuals with a spectrum of phenotypes (a significant number of which were intermediate) and a spectrum of behaviors (a significant number of which were also intermediate) [Beyene, 1998; Bergman, 2000; Beehner, 2003; Beehner et al., 2004; Bergman & Beehner, 2004]. Reproductive success within this zone was described to be maintained by frequency-dependent mating such that males that were the most successful were males that were phenotypically and behaviorally in the majority [Bergman & Beehner, 2003]. In other words, in areas of the zone where the baboon groups were primarily anubis, the anubis males would be most successful; in the areas where the groups were primarily hamadryas, the hamadryas males would be most successful; and in areas in the middle of the zone that are highly hybridized, intermediate or hybrid individuals would have the most success.

In an anubis group in the upstream part of the Awash River, (Group C), immigrant hamadryas males engaged in affiliative interactions with anubis females that were largely unaffected by female reproductive state. However, anubis males showed a marked differential response to estrous females: they were relatively indifferent to non-estrous females, but sharply increased interest and interaction levels when females were estrus [Nystrom, 1992]. Although females followed and groomed hamadryas males more than they did anubis males, suggesting female preference [Nystrom, 1992; Beyene, 1998], when females were in estrus, anubis males were able to monopolize them. This was particularly the case for cycles that resulted in conceptions. As a result, hamadryas males had lower mating success overall than their Anubis-like counterparts [Nystrom, 1992; Beyene, 1998].

In the anubis-yellow hybrid zone in Kenya, where behavioral differences between anubis and yellow baboons were suspected to be small, Tung et al. [2012] found that the zone was characterized by hybrid advantage. Hybrid Anubis-like males matured, dispersed and engaged in more consortship behavior than other males within the group. This resulted in the increased reproductive success of hybrid individuals relative to pure yellow baboons and to the expansion (but dilution) of anubis genes across the zone (thereby contributing to the hybrid asymmetry) [Tung et al., 2008, 2012; Charpentier et al., 2012]. In this zone, they found that the likelihood of consortship (and thus reproductive success) was greater if the male was genetically Anubis-like and dominant, and if members of the pair were not highly genetically divergent (i.e. they were assortatively mating) [Tung et al., 2012].

The kinda-grayfoot hybrid zone shares some features with both the anubis-hamadryas hybrid zone and the anubis-yellow hybrid zone. As in Awash, grooming may be important in the formation and maintenance of the kinda-grayfoot hybrid zone, as it seems that females may prefer (or at least tolerate) males that groom them more. However, this preference does not always result in increased mating success. In Awash, this seemed to be because anubis males increased their affiliative behavior toward estrous, particularly conceptive, females [Nystrom, 1992] and hamadryas males were unable to monopolize their estrous females. In the kinda-grayfoot hybrid group, males that were Kinda-like in **appearance** were also unable to gain access to or monopolize estrous females; however, those that were **genetically** Kinda-like were successful.

Like at Amboseli, I found that dominance rank, mate guarding and male genetic ancestry (male GHIS) were the most influential factors in male mating success. Paternity results from the

Kafue hybrid group were consistent with assortative mating, as no infants were the offspring of parents with GHIS values from both ends of the scale; parents were either strongly genetically Kinda-like fathers with Intermediate mothers (the majority) or strongly Grayfoot-like fathers with Intermediate mothers. Thus, it may be that hybridization and reproductive success in this zone can be best explained by dominant, genetically Kinda-like males mating with females that are not too genetically divergent from themselves.

9.4 Limitations

This study has been a study of a single group within a much larger zone and is a single snapshot in time. As such, it is limited in scope. Since behavioral data from this study were from a single year, this study cannot address questions relating to patterns of hybridization through time (i.e. contraction versus expansion of the zone, hybrid advantage versus hybrid disadvantage, etc.) and requires caution in generalizing the results from this study *across* the hybrid zone. Several hypotheses that were unable to be tested in this study still remain.

9.5 Future Directions

Long-term data from this already habituated group would provide additional life history data that would be useful in assessing how patterns of hybridization change over time. For example, age cohorts and rates and demographics of immigration/emigration of males and births/deaths of infants could be known and compared over time. Longitudinal data would allow mating success to be *directly* compared to parentage of resulting offspring, as well as provide sufficient data to assess differences in mating with regard to conceptive versus non-conceptive

cycles. Finally, results from this study could be further tested and explored. Data on the phenotype and genotype of dominant males as they gain and lose dominance over time would be available, as would data regarding duration of male dominance. In addition, studies could be carried out to try to assess proximate causes for the success of genetically Kinda-like males in obtaining or maintaining dominance. For example, methods could be used to ascertain whether levels of hormones differ among kinda, grayfoot, and hybrid baboons in such a way that might explain why some genetically Kinda-like males are more likely to become dominant and reproductively successful than others.

Long-term behavioral studies of new groups containing different compositions of "pure" and hybrid individuals across the zone (particularly at either end of the zone) would allow for an informative cross-comparative study of behavior. In particular, groups containing some "pure" kinda individuals (individuals who are both phenotypically and genetically Kinda-like) would help address hypotheses that were unable to be thoroughly tested in this study. For example, it is possible that the asymmetry resulted from genetic or obstetric incompatibility between large grayfoot males and small kinda females or that large grayfoot males misinterpreted small kinda individuals in the context of mating and consortship.

Habituating and obtaining behavioral data from a group of "pure" kinda baboons within Kafue National Park is also desirable, as little is currently known about kinda baboon behavior. This additional knowledge would result in a greater understanding of the behavioral differences between kinda and grayfoot baboons and allow me to more appropriately put the results from this study in context of the two species.

9.6 Conclusions

Studies of naturally occurring hybrid zones provide insight into the processes of introgression, reproductive isolation, and speciation. Broad scale studies of hybrid zones can aid in our understanding of the overall size and phenotypic and/or genetic composition of hybrid zones, as well as the ecological differences across the zone which may influence these patterns. Detailed studies of mating behavior in natural hybrid groups within a larger hybrid zone can further reveal phenotypic, genetic, and/or behavioral qualities that are important in the reproductive success of individuals within a group, as well as indicate possible reproductive barriers to hybridization or mate choice [Tung et al., 2012]. Using a combination of macro and micro level studies through time will result in a better overall understanding of the evolutionary and demographic influences shaping hybrid zone dynamics within these populations [Tung et al., 2008]. An overall understanding of hybrid zone dynamics requires knowledge of the rate and direction of gene flow from "pure" animals into the zone, as well as information indicating selective reproductive advantage or disadvantage in individuals within the zone. This study was the first documentation of the behavior and genetics of a kinda-grayfoot hybrid baboon group. This study of a single hybrid group has contributed to our knowledge of patterns and mechanisms of hybridization between kinda and grayfooted chacma baboons. It has revealed that while overall the hybrid zone was primarily formed by kinda male and grayfoot female matings, it was not universally asymmetric. I found that the unusual kinda grooming behavior was a likely factor in the asymmetry and origin of the hybrid zone. Once hybridized, the maintenance of the hybrid zone is the result of a complex interplay of phenotype, genotype, and behavior. In this hybrid group genetically Kinda-like males who are dominant seem to have a reproductive

advantage. Longitudinal studies can help reveal the relationship between Kinda male GHIS and male dominance over time and in other groups. Overall, this study has provided new insight into issues related to mate choice in hybrids, revealed possible reproductive barriers to hybridization, and contributed to our knowledge of baboon hybrid zones, the hybridization of other animals, and baboon diversity in general.

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Appendix 1: Ethanol Precipitation Protocol

1. Put strip tubes of cycle sequencing product in 96-hole base, remove caps.
2. Use electronic digital pipet to aliquot 2.5 µL of 125 mM EDTA di-Na, pH 8.0 into each strip tube.
3. Pour a small amount of 100% ethanol into trough and pull up into multi-tip pipet. Aliquot 30 µL of 100% ethanol to each sample to precipitate DNA. Dispose of remaining 100% ethanol in trough.
4. Cap tubes and mix by inverting 3-4 times (support the tubes so that they don't fall out!). Let mixture set at room temperature for 15 minutes.
5. Place base, with strip tubes, on Eppendorf centrifuge bucket.
6. Centrifuge using program #1 (3000 rcf for 30 minutes) to pellet DNA.
7. Immediately, remove base from bucket, remove caps from tubes. Add additional empty tubes to support towel in next step.
8. Fold towel to base size, place folded towel over tubes, invert tubes and base, and place upside-down onto Eppendorf centrifuge bucket.
9. Centrifuge only up to180 rcf. For the centrifuge program, use B *180. On the Eppendorf centrifuge, an * means rcf.
10. Remove base from bucket, turn right side up, remove paper towel.
11. Pour a small amount of 70% ethanol into trough and pull up into multi-tip pipet. Aliquot 30 µL of 70% ethanol to each sample to wash pellet.
12. Cap tubes, and place base with strip tubes on Eppendorf centrifuge bucket.

13. Centrifuge using program A (3000 rcf for 10 minutes) to pellet DNA.
14. Remove base from bucket, remove caps from tubes. Fold second paper towel, place folded towel over tubes and invert tubes and base, and place upside-down onto Eppendorf centrifuge bucket.
15. Centrifuge using program #8 (1030 rpm for 1 min.) to remove wash supernatant.
16. Immediately remove base from bucket, turn right side up, remove paper towel.
17. Leave the tubes uncapped but covered (to protect DNA from light which can cause degradation) at room temperature for ~15 minutes to ensure that all EtOH has evaporated. If the tubes are not dry, leave them for another 15 minutes. Ethanol can contaminate the capillary array of the 3100 Genetic Analyzer, so make sure all EtOH has evaporated before proceeding. Proceed to genetic analysis preparation or recap the tubes and place tubes in the -20°C freezer.

Appendix 2: Behavioral Ethogram

Behavior Type	Behavior Name	Abbrev.	Description of Behavior
Proximity**	Touch	TO	Sit/stand touching one or more individuals
	0-2 meters	ZT	Sit/stand within 2 meters of another individual (non-contact)
	2-6 meters	TS	Sit/stand within 2-6 meters of another individual
Social	Present Non-sexual	PR	Present hindquarters (non-sexual context)
	Inspect Non-sexual	IN	Inspect after being presented to (non-sexual context)
	Ignore Groom Solicit	IGS	Ignore the groom solicit of another individual
	Approach	AP	Approach another individual (within 2 meters) who then does not move away (within 20 seconds)
	Withdraw	WD	Move away from another individual over 2 meters
	Play behavior	PL	Play with another individual - usually an infant/juvenile
	Social Other	OTS	Any other social behavior not defined in above categories
Affiliative	*Groom	GM	Actively groom another individual
	Solicit Groom	SG	Solicit grooming from another individual
	Grunt	GT	Any of several grunts given in response to infants or friendly encounters
	Affiliative Other	AF	Any other affiliative behavior not defined in above categories
Contact Aggression	Bite	BI	Bite another baboon
	Non-sexual Mount	NSM	Position oneself over another baboon's rear with genitals aligned, hands on torso and standing with hindlimbs on recipient's hindlimbs
	Grabbing	GB	Grabbing another baboon
	Hitting	HI	Hitting another baboon
	Contact Aggression Other	OCA	Any other contact aggression behavior not defined in the above categories
Non-contact	Chase	CH	Run after another baboon (resulting in the other baboon fleeing)
Aggression	Lunge	LU	Sudden but brief movement towards another individual in a threatening manner
	Yawn	YN	Open mouth to full gape, usually facing perpendicular to recipient, threat usually given by adult male
	Eye-flash	EF	Raise eyebrows exposing pink eyelids, threat display
	Displace/Supplant	DP	Individual moves within 2 meters of
	Non-Contact Aggression Other	ONC	Any other non-contact aggression behavior not defined in the above categories

Behavior Type	Behavior Name	Abbrev.	Description of Behavior
Submissive	Fear Grimace	FG	Open mouth and raise lips, often associated with keck
	Tail Lift	TL	Raise tail, exposing perineal region
	Scream	SC	Emit high pictched sound, often in response to threat; any scream vocalizations
	Submissive Other	SU	Any submissive behavior not included in the above categories
Sexual	*Copulate	CO	Intromission not terminating in ejaculation
	*Copulate w/ejaculation	CWE	Copulation terminating with ejaculation
	Follow	FOS	Follow another individuals when in consort
	Sexual Mount	SMO	Mount another individual when in consort - no intromission
	Sexual Present	SPR	Present hindquarters to a male for inspection/copulation
	Sexual Inspect	SIN	Inspect individual after being presented to, tehaviors including sniffing, looking or touching hindquarters - sexual/mating context
	Ignore Present	IP	Not responding to a present (sexual context)
	Sexual Other	OTX	Any other sexual behaviors not defined in above categories
Solitary	Forage	FO	Actively manipulate and/or ingest a foot while traveling; differentiate type (i.e. grass,corms, tubers, fruit, leaves, invertebrates, not visible
	Feed	FD	Actively manipulating and/or ingesting a food; differentiate food type when possible)
	Rest	RT	No movement, sitting, lying or standing
	Horizontal Locomotion	HL	Move/travel on ground
	Vertical Locomotion	VL	Move/travel 1+ meter above ground and/or up/down trees
	Grooms Self	GS	Groom self
	Drink	DR	Drink water
	Solitary Other	OTY	Any other solitary behaviors not listed above
Infant-related Behavior	Aggressive Handling	AH	Aggressive infant handling, including pulling, dragging, biting, etc.
	Carry/Handle Another	CHA	Handle/pick-up/carry another's infant in a gentle affiliative way
	Carry/Handle Own	CHO	Carry or handle own infant

** = all occurrence sampling, as well as continuous focal sampling; ** = 2-min scan sampling*

Appendix 3: *Prim8 Mobile*

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Commentary

'There's an app for that': a new program for the collection of behavioural field data

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Researchers have long sought an inexpensive, reliable, flexible and efficient method to collect behavioural data in the field. Techniques such as free-form notebooks, pre-prepared data sheets and audio/video recordings are inexpensive and require little training but are inefficient, requiring almost as much time to encode the data as to collect it. Conversely, current digital solutions remove the need for data entry after collection, but require users either to purchase commercial software and hardware, or to have the skills and time to design a software solution. This paper introduces a freely available application for the Android operating system, Prim8 Mobile, which can be used for any captive or wild animal study that uses a behavioural sampling protocol combining continuous focal follow, instantaneous scan, all-occurrence and/or ad libitum data. It can also be used to catalogue biological samples, such as animal tissues and faecal or plant matter. This application has been validated for consistency and stability on the Motorola A855 under both controlled and field conditions, including a 12-month field study of baboons in the southern region of the Kafue National Park, Zambia. Advantages of the application include the ability to import and alter protocols on the device, to backup and export the data without internet availability and to use the exported data directly in spreadsheet or statistical analysis software.

Despite a variety of techniques for collecting observational data, the digitization of behavioural data is still impeded by environmental, technical and financial obstacles. To date, the most popular collection methods include free-form notebooks, pre-prepared data sheets, audio recordings, video recordings and electronic data collection devices (Lehner, 1996; Martin & Bateson, 2007), each with benefits and drawbacks. Often, behavioural researchers will use a combination of these sampling methods when observing primates or a variety of taxa (e.g. Alberts & Altmann, 2011; Bayly, Evans, & Taylor, 2006; Bergman et al., 2008; Di Fiore & Rodman, 2001; Engh et al., 2006; Honer et al., 2007; Lynch, Ziegler, & Strier, 2002; Montague, Disotell, & Di Fiore, 2014; Palombit, Seyfarth, & Cheney, 1997; Shaffer, 2013) in addition to cataloguing biological samples (Cortes-Ortiz et al., 2007; Deschner, Heistermann, Hodges, & Boesch, 2004; Montague et al., 2014).

For field researchers specifically, the preferred method of data collection is often dependent upon the collection environment and the researcher's financial resources and/or technological expertise. Recording behavioural data by hand is the least expensive, most flexible and most accessible option, requiring less training and providing the greatest latitude in representing data (Alberts & Altmann, 2011; Bulger, 1993; Hamilton & Bulger, 1990). However, the researcher is limited by the speed at which he or she can write. Moreover, this method requires the researcher to manually time stamp each entry and thus simultaneously manage observing the animal, writing in the notebook and checking a timepiece. If additional field assistants are involved, it also requires training and monitoring their activities to assure that the data collection protocol is being consistently observed (Altmann & Alberts, 2011).

Audio and video recording are not limited by the speed at which one can write and are generally cost effective. Video recording can also allow researchers to score behaviours that would be difficult or impossible to record via other methods and provides a visual record of the behaviour that can substantiate reports and allow researchers to score the data long after collection (Deschner et al., 2004; Sanz & Morgan, 2009). However, video recording is limited by lighting conditions, terrain and animal activity. Also, while improvements in zooming and image stabilization have greatly advanced the usability of video cameras, the devices remain difficult to use effectively while traversing difficult terrain and in

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inclement weather or dusty environments. With all of the aforementioned methods, the researcher must still manually enter (sometimes with the assistance of specialized software) these data into a computer at a later time before data analysis can take place, often doubling the time investment of the research.

A second class of techniques relies on electronic data collection solutions, consisting of data collection software and hand-held computer devices (Ganem, Litel, & Lenormand, 2008). These solutions can provide an efficient means of data collection, often allowing the researcher to export the data directly to a computer for statistical analysis and thereby reducing not only the amount of time and effort between data collection and data analysis but also eliminating common sources of error (Martin & Bateson, 2007). These can additionally improve data standardization by structuring the observer's entries around a specific collection protocol. The currently available solutions, however, require researchers to have purchased an off-the-shelf hardware and software solution or to have designed custom software. The latter is sufficiently difficult and time consuming to preclude its adoption by many; furthermore, the current constrained funding environment makes the commercially available data solutions, such as The Observer (Noldus, 1991), Interact (Mangold, 2010; Martin & Bateson, 2007) and Psion Workabout (Psion Teklogix™), well beyond the financial means of many. Such solutions are often not specifically designed for behavioural data collection, often require hardware that is poorly suited to field conditions or limit the data collection and management protocols one can use (e.g. The Observer; Noldus, 1991). That is, researchers are often faced with the difficult decision of whether to choose an electronic data collection device for efficiency but sacrifice the ability to adopt a sampling scheme that is optimal for his or her study.

This paper details Prim8 Mobile, an application based upon the principles of animal sampling (Altmann, 1974) and the various behavioural sampling regimes commonly discussed in Martin and Bateson (2007) and Lehner (1996). Prim8 Mobile was designed to enable the user to adopt the optimal protocol based on his or her assessment of what can be reliably handled in the working environment. Critically, Prim8 Mobile supports combinations of focal follow, instantaneous scan and all-occurrence behavioural sampling methods and ad libitum data (Altmann, 1974; Lehner, 1996; Martin & Bateson, 2007) on any mobile device with the Android operating system. In addition, it allows the user to import and alter protocols on the device, backup and export the data without internet availability and use the exported data directly in spreadsheet or statistical analysis software.

REQUIREMENTS

The Hardware

Because the software was initially designed to study social primates in remote field conditions, it was necessary to identify a device that was small and rugged, with a relatively long battery life, a simple method for backing-up data and a physical keyboard to assist in nonvisual data entry. To satisfy these requirements, an Android-based device was chosen, as there are many choices for hardware, which are relatively inexpensive and already owned by many researchers (e.g. smart phone, tablet, etc.). Furthermore, these devices can run free applications for voice recording, note taking, photographing and text editing. Unlike some other mobile operating systems, Android-based systems give the user the ability to store files easily using microSD cards and to transfer files via USB and Bluetooth technologies without special software and without requiring internet access. Therefore, the software was designed to work on any device running Android 3 (OS 1.5, Cupcake) or higher, allowing it to be used on both newer and older mobile devices. The testing was completed on the Motorola A855, one such device that fulfils these requirements.

The Software

Conditions vary among research environments, presenting each researcher with different challenges. Important design considerations have included interface economy, optimization of battery life, ease of in-field modifications to protocols (on the device and through editable configuration files) and simple data input and output using file formats that can be interpreted by common statistical and spreadsheet programs.

Although many current applications rely on a touch button interface, Prim8 Mobile has been deliberately designed to require the user to type out short, simple codes (i.e. individual and behaviour abbreviations). There are several reasons for this design choice. First, it mimics the way pencil and paper data are often collected, thus requiring little to no technological learning curve for those migrating from such methods. Second, it allows the application to use fewer graphical elements and incorporate fewer steps for the user. This second justification allows the application to be much less demanding on battery life, allows for maximal study design flexibility and economizes on screen usage to allow the software to fit comfortably on small-screened devices that field researchers can fit into their pockets for freedom of movement. The application was also designed to allow configuration files to be imported into the device and data to be exported from the device as comma-separated value (CSV) formatted files. The CSV format makes the exported files compatible with common spreadsheet (e.g. Microsoft Excel and Numbers) and statistical and data analysis software (e.g. R, SPSS, SAS), drastically reducing both the time spent in collecting and analysing data and eliminating human errors by obviating intermediate data entry steps (Martin & Bateson, 2007). Figure 1 summarizes the workflow in using this software, from data input to data output. The software specifics and formatting standards can be found in the user manual (McDonald & Johnson, 2014). The user manual and the Prim8 Mobile application are freely downloadable at http://www.prim8software.com. The website also contains additional information on the upcoming iPrim8 application, a touch button-based behavioural data collection application for the iOS platform (e.g. iPads, iPods, iPhones, etc.). iPrim8 is currently under development by Prim8 Software and the Lester E. Fisher Center for the Study and Conservation of Apes, and will be offered at no charge to researchers.

Prim8 Mobile was developed in the Java programming language supported by the Android OS and uses SQLite locally for the relational database, which organizes data using a model of how different entities (e.g. individuals, focal follows, researchers, etc.) are related to each other. In this application, the relational database was constructed in a way that ensures that data are stored in a nonredundant way and organized to reduce data errors, while keeping the database as small as possible and making it easier to query.

Prim8 Mobile was designed to support field researchers in general, rather than to be specifically targeted to researchers studying nonhuman primates. The application accommodates behavioural data collection on any taxon (in captive or field environments) where observations include continuous focal follows, focal follows with imbedded scans, all-occurrence and/or ad libitum data. It is optimal for studying activity budgets or social behaviours and directly supports studies involving several different

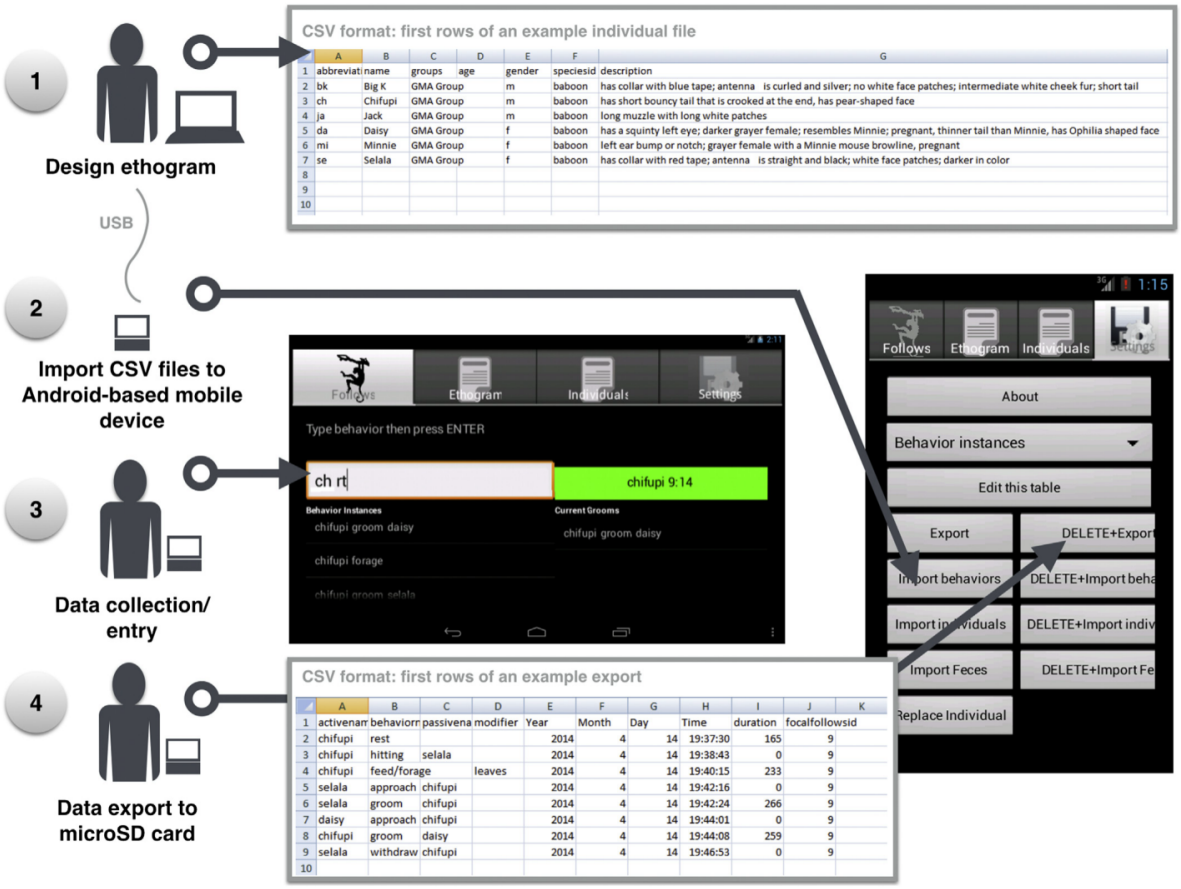

Figure 1. Workflow diagram detailing the designing and uploading of an ethogram, the collection of data and the exporting and format of collected data. Number 3 of this figure is a view of the behaviour collection screen showing the Follow countdown timer and focal individual, a list of focal and all-occurrence behaviours recently entered and the syntax used when recording the behaviour instance 'Chifupi rests'.

sympatric species (or groups) or a single species across many geographical areas. It is also applicable to museum and field studies relating to biodiversity where the collection and cataloguing of biological samples, including plant specimens and tissue, faecal and/or urine samples, is important.

VALIDATION

The software has been designed and tested in three phases. Initial testing was undertaken at the Saint Louis Zoo and the Kansas City Zoo in the spring of 2012 to ensure that the design was practical in a surrogate field situation and to assess the battery life of the device, the ease of data entry and export and to discover any errors in the software. During this initial phase, several issues, such as buzzer malfunctions during scan or focal transitions and the cancellation of follows if the physical keyboard was closed (or if another application was selected during a focal), were identified and subsequently corrected. To ensure that time-based properties of data were being accurately captured, the authors conducted a controlled study, where one researcher entered a script of behaviours and the other independently recorded the durations and time stamps. This confirmed that the application correctly records time stamps, correctly calculates durations for state-based behaviours, interprets behaviours as expected and provides visual and audio cues. Next, the software and selected device (Motorola Droid A855) were further tested and validated during a 12-month field study of baboons in the southern region of the Kafue National Park, Zambia during July 2012–2013 (see Case study below), where McDonald and her field assistant used the device with the Prim8 Mobile application to record the collection of approximately 200 faecal samples and 4500 10-minute focal follows with 2-minute embedded proximity scans, while also recording all occurrence grooming and copulation behaviours and ad libitum data. Additional alterations were made during the field study to allow ad libitum data to be recorded. Prior to this, electronic notes were recorded using a free notepad application on the device. Also, a Replace Individual button was added to allow the user to change the focal animal's name from an unknown (or uncertain) individual at the beginning of a follow to its name once it is recognized. Using the lessons from the field study validation, the software has been augmented with additional

scan and focal time options, buttons and/or tables have been renamed for clarity and the user can now choose buzzer sounds. The current version of Prim8 Mobile, sample template CSV files for assisting users in designing ethograms and the PDF of the user guide are available to download from https://www.prim8software.com.

FEATURES AND USABILITY

Set Up and Inputs

The Prim8 Mobile software can be installed as detailed in the user guide (McDonald & Johnson, 2014). From the Android device, the user presses the Prim8 Mobile icon to start the application. The initial screen, as seen in Fig. 1, is the basic data collection screen with tabs to access details and further modify data associated with the tab names: Follows, Ethogram, Individuals, Settings.

The user begins by creating CSV files. The Individuals file has fields for individual name, abbreviation, description, species, group name, age and sex, while the Ethogram file has fields for behaviour name, abbreviation, description and behaviour type, along with fields to indicate whether or not the behaviour will be a state behaviour, an all-occurrence behaviour and/or a scan behaviour. Once these files are complete and saved as CSV files, the user should populate other associated tables (e.g. Sites and Locations) through the device Settings tab. The application will create default values in these tables upon import of the individuals and ethogram CSV files if the user fails to follow this previous step; however, this is not recommended. The CSV import files can be transferred to the device using USB, e-mail and/or Bluetooth and placed in the main directory of the device's microSD with file names as described in the user manual. To import CSV files, the user selects the Settings tab and clicks the button labelled DELETE + Import Individuals or DELETE + Import Behaviors, respectively. The imported individuals and behaviours can then be viewed in the Individuals and Ethogram tabs, and their descriptions can be viewed by long-pressing the individual or behaviour (Fig. 2).

If the user needs to modify an entry, it can be done directly on the device by choosing the Individuals tab or the Ethogram tab and clicking on the button labelled ADD to add a new entry or a specific individual or behaviour entry for editing purposes (Fig. 2).

Data Collection

Once the individuals, behaviours and necessary relationship tables are populated, the user has a number of options for data collection. The use of the all-occurrence designation for a behaviour allows the user to tailor the application for the user's particular collection protocol. For instance, if a user wishes to take only ad hoc data, all behaviours would be designated all-occurrence; one can additionally enter ad libitum data at any time. If the user wishes to take only scan data, scan and continuous data simultaneously, or only continuous data for a specific period of time, the user can initiate a focal follow. In the case of group scan data, the user creates an 'individual' in their Individuals file named, for example, 'Scan Only', and then selects that for each focal duration (all behaviours would also be set as follow behaviours and scan behaviours).

To start a focal follow, the user selects the Start Follow button in the Follows tab (Fig. 1), then chooses the focal individual, the follow duration and the scan duration (if any), and selects Start Follow. This automatically takes the user back to the data collection screen and displays the countdown timer and focal individual. Next, the user types the abbreviations for the subject, behaviour, recipient(s) and/or modifier(s) into the physical keyboard on the device (Fig. 1).

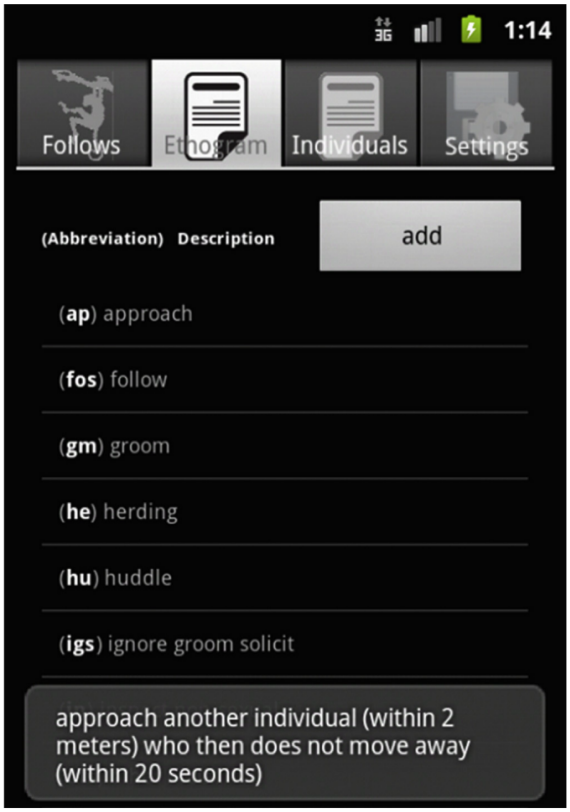

Figure 2. Behaviour and individual names and codes can be viewed in the Ethogram tab and the Individuals tab. This figure illustrates the Ethogram tab. If the user long-presses a behaviour or individual entry, a pop-up box with the description of each will display. Short-clicking will bring up an editing screen.

It is not necessary for the user to type the abbreviations for the follow's focal individual since the program infers this, although one can do so if desired. Once the user has begun to enter the behaviour instance, it will be given a time stamp. Upon hitting the enter key, the behaviour entry will be separated into each of its elements, processed and stored into the database, and translated as an entry in a view list below the text entry box (Fig. 1).

The durations of all state behaviours are calculated automatically and are not interrupted by event behaviours entered concurrently. If a behaviour is entered incorrectly, which can be evaluated by looking at the translated text in the view list, the user can correct it by short-clicking the behaviour in that list at any time. Once selected, the behaviour entry can either be deleted or be re-entered, in which case the original time stamp will be maintained. Behaviours associated with the follow are entered according to the user's specified protocol until the follow buzzer sounds, indicating that the follow has ended. Follows may also be aborted by the user, in which case the cancellation and duration of the cancelled follow will be noted in the database.

If scans are also chosen, a scan buzzer (for which the sound can be specified to be different from the follow buzzer) will periodically sound to alert the user to enter the scan data. All-occurrence behaviour instances can be entered at any time; all-occurrence event behaviours will appear in translated text underneath the text entry box. Any all-occurrence state behaviour instances will

appear in the list view at the right side of the screen and will remain until the user indicates the behaviour has ended by clicking on the entry. Behaviours classified as all-occurrence state behaviours are appropriately handled to maintain the data recording past the end of the focal follow (if needed) in order to capture full durations of specific behaviours as seen in the case study below. Ad libitum data can be entered as notes in the same text box at any time by typing an exclamation point followed by text in any form. These notes will not show up below the text entry box but can be viewed and edited later by choosing Ad Libitum Data in the table view list in the Settings tab screen, then clicking the button labelled Edit the Table.

The combination of physical keyboard and data code entry provides an added benefit in that, with time and experience, the user may record data with less time spent looking at the device and more attention on the individuals. To lend confidence to this reduced attention on the device, users can periodically skim the list of behaviours in translated text to ensure that the behaviours are entered and interpreted as intended and to correct them easily as necessary.

Biological samples can also be easily catalogued using the device. The user selects the Settings tab, Biological Samples from the drop-down menu, then Edit this Table. Next, the user selects Add, then enters the Sample Number, Individual Name, Time of collection, Collector and a Description.

Outputs and Data Modification

Any data entered can be viewed or altered on the device by selecting the appropriate table in the Settings, then pressing the Edit the Table button. When the user is ready to export the data, he or she can select either Export or DELETE + Export. Pressing the Export button will export the data but also retain it in memory, such that additional data will be appended. Pressing the DELETE + Export button will export the data, then delete it from internal memory, so the next data session will only contain new data. In either case, the data are saved onto the microSD card in the device in successively indexed files. Currently, the data are exported as several different CSV files, which are described in more detail in the user manual. Once exported, the data can be easily backed up on a laptop computer and/or portable hard drive by e-mail or by connecting the device to a laptop via USB or Bluetooth and transferring the files; one may also e-mail or upload files to a cloud-based location for remote storage. This can be accomplished with only the device and a wireless internet connection. These choices give the user multiple options for backing up data. The exported files are then ready to be opened in statistical and data analysis packages for further modification and analysis.

Finally, editing of data postcollection is supported through a few different mechanisms. From the behavioural collection screen, a list of recently collected behaviours is displayed, and any behaviour can be selected for re-input (while retaining the original time stamp). The user may also alter data throughout the database as needed by editing individual tables from the Settings tab. There are additional convenience functions to re-input behaviours, including replacing the focal individual for all behavioural instances during a user-specified time frame through a single Replace Individual button on the Settings tab.

CASE STUDY

To illustrate the flexibility of Prim8 Mobile when studying animals with complex and rapid behaviours, we explain the entire procedure described above (Fig. 1) in a case study of field observations on a 64-member group of wild hybrid baboons in southern Kafue National Park, Zambia by McDonald as part of her

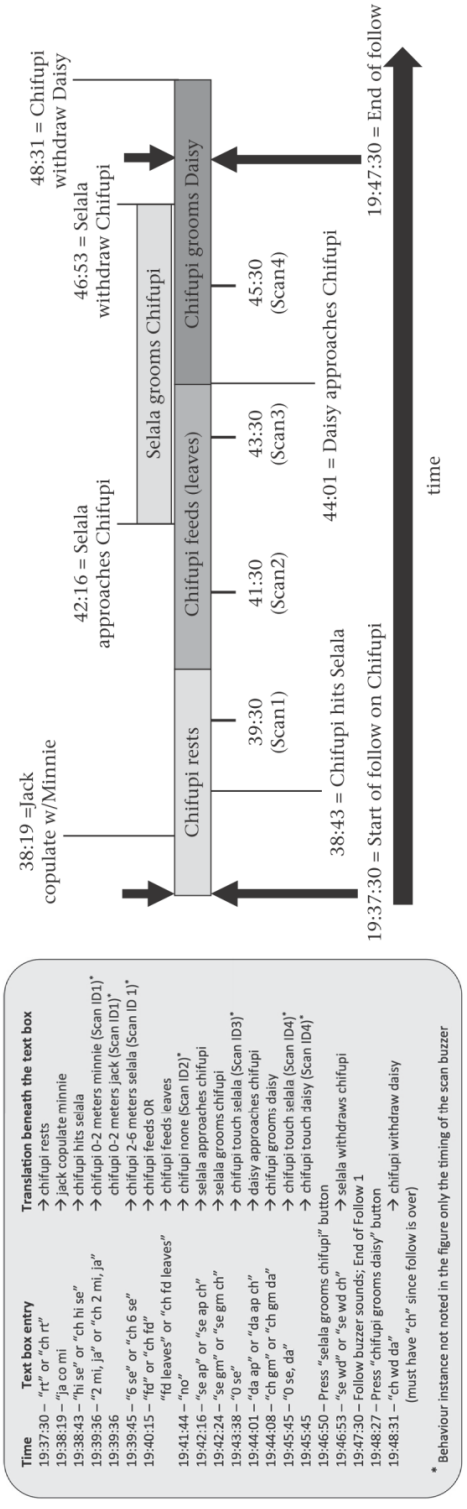

Figure 3. Left: example user entry for the text box followed by the translated text found below the text box after a behaviour is entered. Right: an image illustrating the follow described in the case study. This figure demonstrates support for concurrent collection protocols, allowing the user to take continuous focal follows, all-occurrence and instantaneous scan data during a focal follow.

dissertation research. The study began in July 2012, and addresses questions related to the social behaviour and relative reproductive success of kinda (*Papio kindae*) × chacma (*Papio ursinus griseipes*) hybrid baboons. The protocol consisted of 10 min continuous focal follows with 2 min imbedded proximity scans, all-occurrence grooming and copulation behaviours, and ad libitum data on all identified adult males and females in the group. Faecal samples were also collected from all individuals in the group and catalogued using the software. Data were collected during wet and dry seasons over an approximately 20 km^2 area consisting of woodland, grassland and marsh-like environments.

The necessary relationships tables (e.g. Sites, Location, Behavior Types, Groups, Behavior and Individuals) were populated prior to travelling to the field. Since this baboon group was not habituated to the presence of a human observer at the beginning of the study (i.e. none of the individuals had yet been named), modifications needed to be made in the field as habituation progressed. Behaviours and individuals, including abbreviations, could be added on the device in the field by short-clicking on the behaviour or individual name in the relevant tab (Fig. 2) or by altering the CSV file on the computer at the field site, then transferring the CSV files (see website) to the microSD card in the device. This was accomplished by connecting the device to the computer and importing the files to the device by pressing DELETE + Import Individuals and DELETE + Import Behaviors in the Settings tab. These individuals and behaviours, along with abbreviations and descriptions, could then be referenced by selecting the Individuals tab or the Ethogram tab (Fig. 2).

The individual and ethogram CSV files that were imported to create the follow described below (and illustrated in Fig. 3) can be viewed on the website. In this example, 'grooming' (gm) is an all-occurrence state behaviour, the behaviours 'rest' (rt) and 'forage' (fo) are state behaviours, and 'hit' (hi), 'approach' (ap) and 'withdraw' (wd) are event behaviours. Scan data include all proximity categories. Next to the illustration in Fig. 3, the time stamps, user-entered text and its translation by the application are listed. Durations were automatically calculated for the mentioned state behaviours. The event behaviours did not interrupt any duration calculations.

The follow (Fig. 3) begins with pressing Start Follow in the Follows tab and choosing 'Chifupi' as the focal individual, '10 minutes' as the focal follow duration and '2 minutes' as the imbedded scan duration. Then the Start Follow button is pressed, and the 10 min follow immediately begins. Since copulations are all-occurrence event behaviours, Jack (ja) is recorded as copulating (co) with Minnie (mi), even though neither of these individuals are the focal individual. This entry does not disrupt the duration of 'Chifupi rests'. Two minutes into the follow, and every 2 min thereafter for the duration of the follow, a scan buzzer sounds. Between the first and second scan buzzer, proximity information relative to the focal individual is entered. For example, for Scan ID1, Minnie and Jack are less than 2 m apart (so '2' is entered), and Selala (se) is 2–6 m from Chifupi (so '6' is entered), while Chifupi is resting. The two proximity entries will have different time stamps because they are two different entries. However, since the user designated the behaviours to be 'scan' behaviours, and they have been typed before the next scan buzzer, both instances are assigned to Scan ID1. Selala (se) approaches (ap) Chifupi and begins grooming him while he is still feeding. Since grooming is an all-occurrence state behaviour, this behaviour moves to the right side of the screen to note that it is in progress, and the duration is calculated for it. Daisy (da) approaches Chifupi, and he begins grooming (gm) her. Since he is the focal individual, this ends the 'feeding' duration and begins a 'grooming' duration for him. Because it is a grooming behaviour, it also moves to the right side of the screen underneath the other grooming behaviour. Selala withdraws (wd) from Chifupi, which is recorded by pressing the appropriate grooming instance on the right side of the screen, which also ends its duration. The Chifupi/Daisy grooming bout continues even after the follow buzzer sounds. Follow 1 has ended, yet because groom is an all-occurrence state behaviour, its duration continues past the end of the follow, and the end of the grooming duration is signalled by pressing the grooming entry on the right side of the screen when Daisy withdraws (wd) from Chifupi. To determine approach and withdraw information associated with grooms, the entry also indicates that Daisy withdrew. In this case, since the behaviour is outside of a follow, both individuals' abbreviations are entered, since the focal individual can no longer be inferred.

The data are exported by selecting the Settings tab and by pressing the Export button. Resultant files are then examined using a free text editor on the device to verify visually that they have been exported to the microSD card. These files are treated as intermediate backups for the data up to that point in the week. The next day's data will then be appended to that of the previous day. An illustration of the layout for the exported data output from the follow described in Fig. 3 is available on the website. At the end of a week, the full data set for the week will be exported by clicking the button labelled DELETE + Export (rather than Export), which will expunge the data from the application after writing the CSV files. The exported files will then be copied from the microSD card to a laptop. The original exported data are retained but then edited in Microsoft Excel every 2 weeks (saving it with a different file name) to address and integrate any applicable ad libitum notes that are taken over this time frame.

Summary

Prim8 Mobile is a freely available Android OS-based application that provides a flexible, customizable and efficient method to collect behavioural data and catalogue biological samples for captive and wild taxa in a variety of contexts. Addressing the constraints of other data collection devices, Prim8 Mobile supports any combination of continuous focal follow, instantaneous scan, all-occurrence and ad libitum data. There are a multitude of choices for supported hardware, and many researchers may already own the necessary hardware (e.g. Android-based smart phone, tablet, etc.). The application allows the researcher to upload individuals and ethograms quickly and easily, either collectively via CSV-formatted files or manually on the device while in the field. The data entry syntax is similar to the way pencil and paper data is collected, thus requiring little to no learning curve. Finally, it allows the user to export the digitally collected data as CSV-formatted files that are ready to be opened in statistical and data analysis packages (e.g. R, SPSS, SAS, etc.), drastically reducing the time spent in the data entry and analysis phases of research. It also eliminates some sources of human error by obviating these intermediate data entry steps. The application not only makes data collection and analysis easier for a solitary researcher, it also provides a standardized approach to data collection protocols and file format that can facilitate cross-comparative behavioural studies across research groups.

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Appendix 4: Pregnancy and Lactation Details

1. Arwen: Her infant's status is unknown from 1/12/2013-2/20/2013; assumed to be black infant.

2. Daisy: Her infant status is unknown from 9/18/2013-10/18/2013; assumed to still be black infant.

3. Jean: Birth date of infant is uncertain; could have been between 9/1/2013 and 10/15/2013; date shown in group is from counted up from deflating day on last estrus cycle

4. Ndona: Birth date of infant is uncertain; baby appeared to be recently born on 11/8/2013 though date shown in the graph is from counting up from deflating day on last estrus cycle.

5. Ophilia: Birth date of infant was predicted for 12/17/2013 but no infant was ever seen.

6. Rosy: Birth of infant (or miscarriage) must have occurred between 8/29/2012 and 10/15/2013; she was still observed to be in her their trimester on 8/29/2013 but was flat with no baby on 10/15/2013.

7. Tamanga: She and her baby disappeared shortly after 3/10/2013 and were never seen again.

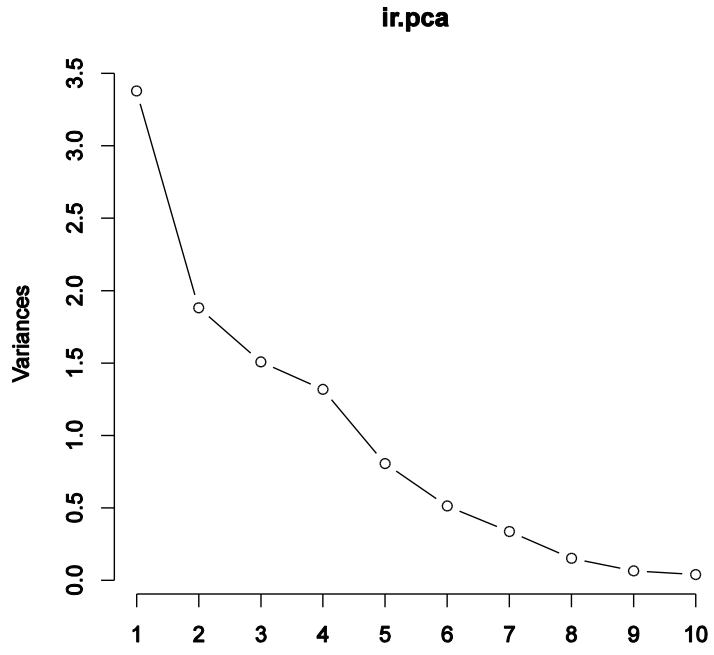
8. Yin: Her birth was predicted for 12/1/2013; not sure when birth actually happened but there was a healthy offspring born.

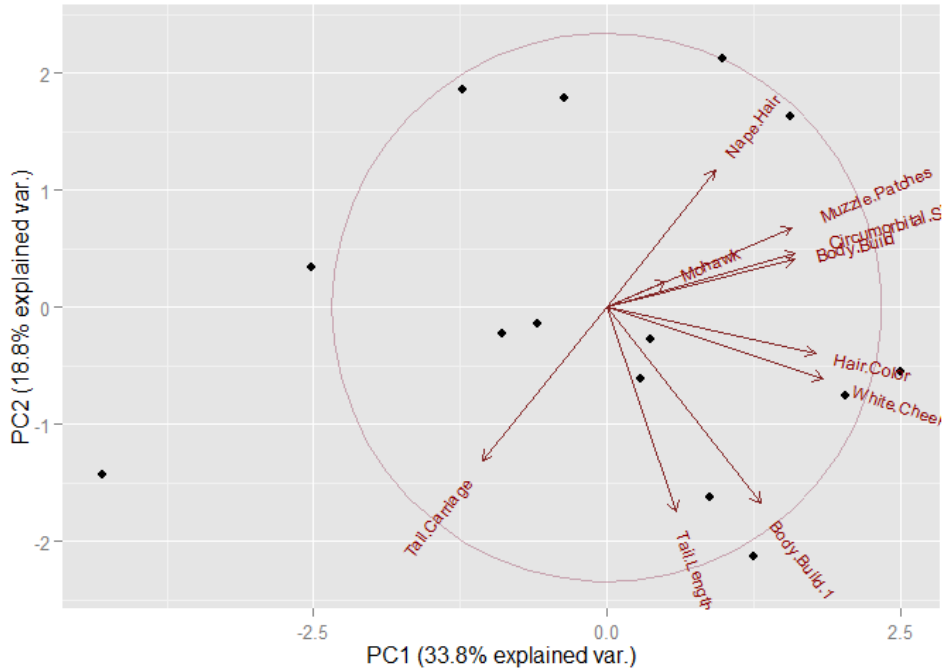
9. Flora: She had a new infant when she was first observed and identified, so pregnancy stages are estimated from this; no prior observation of pregnancy was observed.

10. Helena: She had a new infant when she was first observed and identified, so pregnancy stages area estimated from this; no prior observation of pregnancy was observed.

11. Zelda: She was observed back in estrus on 10/15/2013.

Appendix 5: PCA Analysis of Phenotypic Features





Standard deviations:

1.84	1.37	1.23	1.15	0.90	0.72	0.58	0.39	0.26	0.20

Rotation:

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
MuzzlePatches	0.37	0.21	-0.26	-0.42	0.24	-0.05	-0.14	0.69	-0.04	-0.16
NapeHair	0.21	0.37	-0.09	0.33	0.62	0.48	0.00	-0.24	-0.17	0.07
Mohawk	0.12	0.07	0.70	-0.12	-0.28	0.48	-0.09	0.26	-0.25	0.20
CircumorbitalSkin	0.37	0.15	0.07	-0.54	-0.02	-0.30	-0.03	-0.56	-0.36	0.11
TailCarriage	-0.25	-0.41	0.29	-0.06	0.51	-0.18	-0.62	-0.01	-0.12	-0.06
TailLength	0.14	-0.54	0.07	-0.35	0.34	0.23	0.50	-0.02	0.30	0.21
HairColor	0.41	-0.12	0.07	0.45	0.04	-0.44	0.01	0.21	-0.05	0.61
BodyBuild	0.37	0.13	0.51	0.18	0.10	-0.25	0.12	-0.07	0.39	-0.56
BodyBuild.1	0.30	-0.52	-0.17	0.25	-0.14	0.12	0.11	0.03	-0.56	-0.43
WhiteCheekFur	0.43	-0.19	-0.23	0.00	-0.27	0.32	-0.55	-0.20	0.45	0.06

Importance of components:

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Standard deviation	1.84	1.37	1.23	1.15	0.90	0.72	0.58	0.39	0.26	0.20
Proportion of Variance	0.34	0.19	0.15	0.13	0.08	0.05	0.03	0.02	0.01	0.00
Cumulative Proportion	0.34	0.53	0.68	0.81	0.89	0.94	0.97	0.99	1.00	1.00

Appendix 6: Behavioral Data

Male ID	Grooming Duration (in seconds)	Total Mate Guarding (# of instances)	Peak Mate Guarding (# of instances)	Total Copulations (# of instances)	# of offspring fathered
Big K	6482	84	10	93	0
Chifupi	2921	74	19	92	0
Jack	974	36	7	23	1
Kink	4134	22	6	11	1
Kuyipa	0	0	0	4	0
Mavuto	16	7	1	4	0
R. Simmons	0	5	0	16	2
Solomon	1651	18	13	19	3
Spock	4985	45	26	46	1
Strider	8520	301	96	228	5
Sub Males	3132	7	1	213	0
Uno	198	8	1	8	0
Valentino	114	0	1	60	0
Yogi	158	3	0	11	0
Total	**33285**	**610**	**181**	**828**	**13**
# follows/ behavior*	184	610	181	534	NA

Grooming Duration = Number of total seconds each male was observed grooming a female, regardless of reproductive condition.
Total Mate Guarding =Total number of follows in which each male was observed to mate guard a female, regardless of reproductive condition.
Peak Mate Guarding = Total number of follows in which each male was observed to mate guard a peak estrous female.
Total Copulations = Total number of instances each male was observed to copulate with a female
# of offspring fathered = Total number of offspring each male fathered.
** The number of follows in which each behavior was observed; all data in this table are behaviors carried out by males during female focal follows, as described in Chapter 6.*

Appendix 7: Data Used in GLMM Analysis

Observ	Male	Female	Cop_Peak	M_Rank_DS	MG_Peak	GrmMin_ALL	M_PHIS	M_GHIS	F_PHIS	F_GHIS	Foll_Peak
1	Big K	April	2	7.25	5	35.57	0.15	0.57	0.59	0.11	111
2	Big K	Jesmine	0	7.25	0	1.49	0.15	0.57	0.5	NA	57
3	Big K	Merry	0	7.25	0	0	0.15	0.57	0.77	NA	58
4	Big K	Ndona	0	7.25	0	44.17	0.15	0.57	0.91	0.31	68
5	Big K	Ophilia	3	7.25	5	0.85	0.15	0.57	0.77	0.27	68
6	Big K	Yin	0	7.25	0	20.97	0.15	0.57	0.45	0.47	52
7	Chifupi	April	26	6.04	19	25.8	0.24	0.38	0.59	0.11	111
8	Chifupi	Jean	0	6.04	0	3.87	0.24	0.38	0.59	0.2	17
9	Chifupi	Merry	0	6.04	0	0	0.24	0.38	0.77	NA	58
10	Chifupi	Ndona	1	6.04	0	0	0.24	0.38	0.91	0.31	68
11	Chifupi	Ophilia	0	6.04	0	1.15	0.24	0.38	0.77	0.27	68
12	Chifupi	Rosy	0	6.04	0	9.45	0.24	0.38	0.5	0.09	0
13	Chifupi	Yin	0	6.04	0	0	0.24	0.38	0.45	0.47	52
14	Jack	Jean	0	6.3	6	12.65	0.84	0.06	0.59	0.2	17
15	Jack	Ndona	0	6.3	0	0	0.84	0.06	0.91	0.31	68
16	Jack	Ophilia	1	6.3	1	0	0.84	0.06	0.77	0.27	68
17	Jack	Yin	1	6.3	0	0	0.84	0.06	0.45	0.47	52
18	Kink	April	3	6.11	5	13.14	0.04	0.93	0.59	0.11	111
19	Kink	Ndona	0	6.11	0	47.04	0.04	0.93	0.91	0.31	68
20	Kink	Ophilia	1	6.11	1	0	0.04	0.93	0.77	0.27	68
21	Kink	Yin	1	6.11	0	0	0.04	0.93	0.45	0.47	52
22	Kuyipa	April	0	7.71	0	0	1	NA	0.59	0.11	111
23	Kuyipa	Jean	0	7.71	0	0	1	NA	0.59	0.2	17
24	Kuyipa	Yin	0	7.71	0	0	1	NA	0.45	0.47	52
25	Mavuto	Jean	0	6.8	0	0	0.93	0.14	0.59	0.2	17
26	Mavuto	Merry	0	6.8	0	0	0.93	0.14	0.77	NA	58
27	Mavuto	Ophilia	1	6.8	1	0	0.93	0.14	0.77	0.27	68
28	Mavuto	Yin	0	6.8	0	0	0.93	0.14	0.45	0.47	52
29	R. Simmons	Ndona	0	7.18	0	0	0.53	0.98	0.91	0.31	68
30	Solomon	Merry	6	7.18	12	27.35	0.95	0.02	0.77	NA	58
31	Solomon	Ophilia	1	6.86	1	0.17	0.95	0.02	0.77	0.27	68
32	Spock	Gaga	0	6.05	0	0	0.84	0.88	0.59	0.07	0
33	Spock	Merry	3	6.05	2	10.47	0.84	0.88	0.77	NA	58

34	Spock	Ndona	21	6.05	24	64.4	0.84	0.88	0.91	0.31	68
35	Spock	Yin	0	6.05	0	3.42	0.84	0.88	0.45	0.47	52
36	Strider	April	27	9.45	28	45.49	0.33	0.03	0.59	0.11	111
37	Strider	Merry	9	9.45	13	1.24	0.33	0.03	0.77	NA	58
38	Strider	Ndona	23	9.45	19	53.1	0.33	0.03	0.91	0.31	68
39	Strider	Ophilia	2	9.45	9	5.85	0.33	0.03	0.77	0.27	68
40	Strider	Yin	16	9.45	27	35.19	0.33	0.03	0.45	0.47	52
41	Sub Male	April	3	4.46	1	0	0.18	0.05	0.59	0.11	111
42	Sub Male	Gaga	0	4.46	0	0	0.18	0.05	0.59	0.07	0
43	Sub Male	Jean	3	4.46	0	0	0.18	0.05	0.59	0.2	17
44	Sub Male	Jesmine	8	4.46	0	20.6	0.18	0.05	0.5	NA	57
45	Sub Male	Merry	2	4.46	0	5.32	0.18	0.05	0.77	NA	58
46	Sub Male	Ndona	2	4.46	0	0	0.18	0.05	0.91	0.31	68
47	Sub Male	Ophilia	8	4.46	0	19.04	0.18	0.05	0.77	0.27	68
48	Sub Male	Yin	0	4.46	0	6.22	0.18	0.05	0.45	0.47	52
49	Uno	April	0	5.35	0	0	0	0.31	0.59	0.11	111
50	Uno	Jean	0	5.35	0	0	0	0.31	0.59	0.2	17
51	Uno	Merry	0	5.35	0	0	0	0.31	0.77	NA	58
52	Uno	Ndona	2	5.35	1	0	0	0.31	0.91	0.31	68
53	Uno	Ophilia	1	5.35	0	0	0	0.31	0.77	0.27	68
54	Valentino	April	2	4.27	0	1.27	0	0.74	0.59	0.11	111
55	Valentino	Gaga	0	4.27	0	0	0	0.74	0.59	0.07	0
56	Valentino	Jesmine	4	4.27	0	0	0	0.74	0.5	NA	57
57	Valentino	Merry	0	4.27	0	0	0	0.74	0.77	NA	58
58	Valentino	Ndona	0	4.27	0	0.64	0	0.74	0.91	0.31	68
59	Valentino	Ophilia	4	4.27	1	0	0	0.74	0.77	0.27	68
60	Yogi	Jean	1	7.18	0	0	0.35	0.12	0.59	0.2	17
61	Yogi	Merry	0	7.18	0	0	0.35	0.12	0.77	NA	58
62	Yogi	Ndona	0	7.18	0	0	0.35	0.12	0.91	0.31	68
63	Yogi	Ophilia	0	7.18	0	0	0.35	0.12	0.77	0.27	68
64	Yogi	Yin	0	7.18	0	0	0.35	0.12	0.45	0.47	52

Observation = variable used to account for overdispersion
Male = Male ID/Name
Female = Female ID/Name
Cop_Peak = # of copulations with female when she was peak estrous
M_Rank_DS = Male's Raw David's Score Dominance Rank
MG_Peak = # of follows male mate guarded female when she was peak estrous
GrmMin_ALL = # of minutes in which male groomed female (regardless of reproductive state)
M_PHIS = Male's Phenotypic Hybrid Index Score
M_GHIS = Male's Genetic Hybrid Index Score
F_PHIS = Female's Phenotypic Hybrid Index Score
F_GHIS = Female's Genetic Hybrid Index Score
Foll_Peak = # of follows male was with female when she was peak estrous
All data were from female focal follows as described in Chapter 6